CHRONIC TOXICITY SUMMARY

CADMIUM AND CADMIUM COMPOUNDS

CAS Registry Number: 7440-43-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.01 μg/m^3** (respirable)
Critical effect(s)	Kidney effects (proteinuria) and respiratory effects (reduction in forced vital capacity and reduction in peak expiratory flow rate) in occupationally exposed humans
Hazard index target(s)	Kidney; respiratory system

II. Physical and Chemical Properties (ATSDR, 1993)

Description	Blue-white solid
Molecular formula	Cd
Molecular weight	112.41 g/mol
Specific gravity	8.642 @ 20ºC
Boiling point	767ºC
Melting point	320.9ºC
Vapor pressure	1 torr @ 394ºC
Conversion factor	Not applicable

III. Major Uses or Sources

The production of nickel-cadmium batteries is currently the primary use of cadmium (ATSDR, 1993). The by-product of zinc- and sulfide-ore processing, cadmium is also used for metal plating and in pigments and plastics.

IV. Effects of Human Exposure

Pulmonary and renal function were examined in three worker groups: women with less than 20 years of exposure [E1], men with less than 20 years of exposure [E2], and men with more than 20 years of exposure [E3] (Lauwerys *et al.*, 1974). Although urine cadmium concentrations were significantly elevated, the subjects in E1 did not exhibit pulmonary function changes or proteinuria indicative of renal impairment. The workers in E1 had been exposed for a mean of 4.08 years to 31 μg/m^3 total cadmium (1.4 μg/m^3 respirable cadmium). The 27 workers of E2 had been exposed for a mean of 8.6 years to 134 μg/m^3 total cadmium (88 μg/m^3 respirable

cadmium). The blood and urinary cadmium levels of these workers were also significantly elevated compared to matched controls. Glomerular proteinuria was observed in 15% of the workers in E2 and in 68% of workers in E3. The 22 workers of E3 had been exposed for a mean of 27.8 years to 66 $\mu g/m^3$ total cadmium (21 $\mu g/m^3$ respirable cadmium). Significantly increased levels of cadmium were observed in the blood and urine and workers in E3 also exhibited significant decreases in some measures of pulmonary function (forced vital capacity, forced expiratory volume in one second, and peak expiratory flow rate). This study identifies the kidney as the key target organ of chronic cadmium exposure. For respirable cadmium, this study indicates a LOAEL of 21 $\mu g/m^3$ for workers exposed for 28 years and a NOAEL of 1.4 $\mu g/m^3$ for workers exposed for 4 years.

A study of 82 cadmium exposed workers reports the time-weighted cumulative exposure index (TWE) and cadmium body burden determined in vivo (Ellis *et al.*, 1985). Evidence of renal dysfunction (usually elevated urinary β_2-microglobulin) was consistently observed when the worker's liver cadmium burden exceeded 40 ppm and the time-weighted cumulative exposure index exceeded 400-500 μg years/m^3.

A detailed investigation of renal function in 75 male cadmium-exposed workers identified significant increases in urinary excretion of several low- and high molecular weight proteins, including β_2-microglobulin, and significant decreases in renal reabsorption of calcium, urate, and phosphate compared to controls (Mason *et al.*, 1988). Exposures, which ranged from 36-600 $\mu g/m^3$, were determined from background or personal exposure measurements made between 1964 and 1983, or were estimated. A time-weighted cumulative exposure index (TWE) was determined for each subject. A two phase linear regression model was applied to the data to identify inflection points for each biochemical parameter. The biochemical indicators most highly correlated to exposure were urinary retinol binding protein and urinary β_2-microglobulin. Of these, the most sensitive parameter, urinary β_2-microglobulin, demonstrated an inflection point at 1108 μg years/m^3 with a 95% lower confidence limit of 509 μg years/m^3. The endpoint selected is indicative of defects in tubular reabsorption of proteins.

Diminished sensitivity of smell has also been observed in cadmium exposed workers (Rose *et al.*, 1992). Cadmium body burden, β_2-microglobulin levels, and olfactory function were measured in a group of 55 male workers exposed to cadmium fumes in a brazing operation. A group of 15 control workers was also tested. Exposed workers exhibited high urinary cadmium levels, tubular proteinuria and a significant, selective defect in odor detection threshold.

V. Effects of Animal Exposure

Interstitial infiltration of lymphocytes and leukocytes and hyaline casts were observed in the kidneys of rabbits following exposure to 6.5 mg/m^3 cadmium-iron dust for 3 hours per day, 21 days per month for 9 months (Friberg, 1950). Proteinuria was observed in the majority of exposed rabbits by the fourth month of exposure. Increased lung weights and emphysema were also observed. The trachea and nasal mucous membranes exhibited chronic inflammatory

changes (not specified) and lymphocyte infiltration. The kidney contained the greatest concentration of cadmium. This study also exposed a group of rabbits to 9.1 mg/m^3 cadmium-iron dust for 3 hours per day, 23 days per month, for 7 months. Two rabbits in this group died from acute pneumonia at one month, and one rabbit was sacrificed at 3 months of exposure. Findings at necropsy were similar, although more severe than those observed in rabbits exposed to 6.5 mg/m^3. Chronic bronchitis and hyperplasia of the bronchiolar epithelium were observed in the higher dose group in addition to the findings previously noted.

Male and female rats were exposed to 0, 0.3, 1.0, or 2.0 mg Cd/m^3 (as $CdCl_2$) 6 hours per day, 5 days per week for a total of 62 exposures (Kutzman *et al.*, 1986). Rapid, shallow breathing and marked weight loss were observed in the highest dose group; all animals in this group died within the first 45 days of exposure. A dose-dependent increase in lung weight was observed in the remaining dose groups and a statistically significant increase in lung collagen and elastin was observed in rats exposed to 1.0 mg/m^3. Pathological changes noted in the terminal bronchioles include flattening and hyperplasia of type II cells, and infiltration of macrophages, mononuclear cells, and polymorphonuclear leukocytes. Proliferation of fibroblasts with deposition of collagen was also noted.

Male rats were exposed continuously to 0, 30, or 90 µg Cd/m^3 cadmium oxide (CdO) dust for up to 18 months (Takenaka *et al.*, 1990). Animals exposed to 30 µg/m^3 were sacrificed at 6 and 18 months of exposure. Although some rats in the high dose group were sacrificed after 6 months of exposure, the remaining rats were terminated after 7 months due to increased mortality and were not included in the study. Inflammation and hyperplasia of the alveolar epithelium occurred in animals of both groups after 6 months of exposure with more marked changes observed in the high dose group. Abnormal proliferation of the epithelium was observed in the low dose group following 18 months of exposure. Lung tumors observed in both dose groups were characterized as being duration dependent.

VI. Derivation of Chronic Reference Exposure Levels (REL)

Derivation of Chronic Inhalation Reference Exposure Level

Study	Lauwerys *et al.*, 1974
Study population	Humans (22 exposed men and 22 unexposed men in LOAEL group; 31 exposed women and 31 non-exposed women in NOAEL group)
Exposure method	Occupational exposures
Critical effects	Kidney effects - proteinuria in 68% of LOAEL group Respiratory effects - Reduction in forced vital capacity over 1 second; reduction in peak expiratory flow rate
LOAEL	21 µg/m^3 respirable cadmium

NOAEL	1.4 μg/m^3 respirable cadmium
Exposure continuity	Assumed to be 8 hours/day for 5 days/week
Average occupational exposure	0.33 μg/m^3 for NOAEL group
Human equivalent concentration	0.33 μg/m^3 for NOAEL group
Exposure duration	Average of 4.1 years (1 to 12 years) for NOAEL group
Subchronic uncertainty factor	3
LOAEL uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.01 μg/m^3

This evaluation is strengthened by being based on a human exposure study of workers exposed to cadmium for over 20 years. The exposed group was matched to a control group in terms of age, body size, cigarettes smoked per day and duration of smoking, and duration of employment. The factory process was unchanged over the study period suggesting that exposures may have remained relatively consistent over time.

Significant areas of uncertainty include an incomplete knowledge of the past exposures over the full study interval and the relatively small study size.

A similar evaluation of the LOAEL group led to an alternative inhalation reference exposure level estimate of 0.05 μg/m^3. The LOAEL group had an average occupational exposure of 5.0 μg/m^3 and an average exposure duration of 27.8 years (21 to 40 years). Default uncertainty factors would include a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor (UF).

Using data presented by Ellis and associates (1985) and Mason and associates (1993) correlating human cumulative exposures (in terms of μg-years / m^3) and renal tubular protein reabsorption, a LOAEL of 500 μg-years / m^3 was predicted. This correlates to 7 μg/m^3 over 70 years. A time-weighted exposure to account for continuous exposure rather than 40 hour per week occupational exposure is 1.7 μg/m^3. Applying a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor results in a REL value of 0.02 μg/m^3.

Derivation of Chronic Oral Reference Exposure Level

Study	U.S. EPA, 1985
Study population	Humans
Exposure method	Food and drinking water
Critical effects	Significant proteinuria
LOAEL	Not observed
NOAEL	0.005 mg/kg bw-day

Exposure continuity	Chronic
Exposure duration	Up to lifetime
Average exposure	0.005 mg/kg bw-day
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies factor	10
Cumulative uncertainty factor	10
Oral reference exposure level	0.0005 mg/kg bw-day

The oral REL is the U.S. EPA's Reference Dose (RfD) (IRIS, 1996). A concentration of 200 ug cadmium (Cd)/gm wet human renal cortex is the highest renal level not associated with significant proteinuria (U.S. EPA, 1985). A toxicokinetic model is available to determine the level of chronic human oral exposure (NOAEL) which results in 200 ug Cd/gm wet human renal cortex; the model assumes that 0.01% day of the Cd body burden is eliminated per day (U.S. EPA, 1985). Assuming 2.5% absorption of Cd from food or 5% from water, the toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 0.005 and 0.01 mg Cd/kg/day from water and food, respectively (i.e., levels which would result in 200 ug Cd/gm wet weight human renal cortex). Thus, based on an estimated NOAEL of 0.005 mg Cd/kg/day for Cd in drinking water and an UF of 10, an RfD of 0.0005 mg Cd/kg/day (water) was calculated; an equivalent RfD for Cd in food is 0.001 mg Cd/kg/day.

Cd is unusual in relation to most, if not all, of the substances for which an oral RfD has been determined in that a vast quantity of both human and animal toxicity data are available. The RfD is based on the highest level of Cd in the human renal cortex (i.e., the critical level) not associated with significant proteinuria (i.e., the critical effect). A toxicokinetic model has been used to determine the highest level of exposure associated with the lack of a critical effect. Since the fraction of ingested Cd that is absorbed appears to vary with the source (e.g., food vs. drinking water), it is necessary to allow for this difference in absorption when using the toxicokinetic model to determine an RfD.

The uncertainty factor of 10 is used to account for intrahuman variability to the toxicity of this chemical in the absence of specific data on sensitive individuals. No modifying factor was used.

U.S. EPA stated its confidence in the RfD as: Study - Not applicable; Data Base - High; and RfD -- High The choice of NOAEL does not reflect the information from any single study. Rather, it reflects the data obtained from many studies on the toxicity of cadmium in both humans and animals. These data also permit calculation of pharmacokinetic parameters of cadmium absorption, distribution, metabolism and elimination. All of this information considered together gives high confidence in the data base. High confidence in either RfD follows as well.

VII. References

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CHRONIC TOXICITY SUMMARY

CARBON DISULFIDE

(Synonym carbon bisulfide; carbon sulfide; dithiocarbonic anhydride)

CAS Registry Number: 75-15-0

I. Chronic Toxicity Summary

Inhalation reference exposure level	**700 µg/m^3** (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	CNS/PNS (Reduction in motor nerve conduction velocities in occupationally-exposed humans)
Hazard index target(s)	Nervous system; reproductive system

II. Physical and Chemical Properties Summary (HSDB, 1995)

Molecular formula	CS_2
Molecular weight	76.14 g/mol
Description	Clear, colorless or faintly yellow liquid
Vapor pressure	297 mm Hg @ 20^{o}C
Solubility	Slightly soluble in water (2.94 g/L); miscible in anhydrous methanol, ethanol, ether, benzene, chloroform, and carbon tetrachloride
Conversion factor	3.1 mg/m^3 per ppm at 25^{o}C

III. Major Uses and Sources

The most prominent industrial use of carbon disulfide is in the production of viscose rayon fibers. Other uses include in the production of carbon tetrachloride and cellophane, and, as a solvent for rubber, sulfur, oils, resins and waxes. In the past, carbon disulfide was used in soil fumigation and insect control in stored grain. Industrial processes that produce carbon disulfide as a by-product include coal blast furnaces and oil refining (HSDB, 1995).

IV. Effects of Human Exposure

A primary target of carbon disulfide (CS_2) toxicity is the nervous system. The major neurotoxic action of carbon disulfide is the development of mental disturbances, such as change of personality, irritability, and forgetfulness, often with accompanying neurophysiological and

neuropathology changes after prolonged exposure (decreased peripheral nerve impulse conduction, motor and/or sensory neuropathies, cerebral or cerebellar atrophy, and, neuropsychological organic changes) (Aaserud *et al.* 1988, 1990, 1992; Foa *et al.*, 1976; Hirata *et al.* 1991; Ruijten *et al.* 1990, 1993). Alterations in behavioral indices have been historically associated with high levels of CS_2, often in the excess of 20 ppm (Foa *et al.* 1976; Hannien *et al.*, 1978).

Studies have identified alterations in the nerve conduction of workers chronically exposed to lower CS_2 levels (Hirata *et al.*, 1992; Johnson *et al.*, 1983; Ruijten *et al.*, 1990; Ruijten *et al.*, 1993). A cross-sectional study of Japanese spinning workers identified alterations in the central nervous system as measured by brain stem auditory evoked potential (BAEP) (Hirata *et al.*, 1992). The latencies of the three main BAEP components increased significantly in the CS_2 exposed workers (more than 20 years duration) when compared to controls. CS_2 exposures ranged from 3.3 to 8.2 ppm (mean 4.76 ppm). Ruijten *et al.* (1993) identified mild presymptomatic nerve impairment (decreased conduction velocities and response amplitudes) in 44 CS_2-exposed workers with an average cumulative exposure range 192 to 213 ppm-year (mean duration 26.1 years).

In another occupational study evaluating the effects of CS_2 exposure on the peripheral nervous system, Johnson *et al.* (1983) identified a significant dose related reduction in the motor nerve conduction velocities in the calves and ankles of workers exposed to high (median 7.6 ppm) CS_2 levels versus a comparison group (median 0.2 ppm). Since this motor nerve reduction was still within normal values, the authors considered the measured difference an indication of minimal neurotoxicity. The mean exposure concentration for all exposed workers (n = 145) ranged from 0.6 to 16 ppm (mean 7.3 ppm) with a mean 12.1 year duration. This study established a chronic LOAEL of 7.6 ppm for minor neurological effects (decreased peroneal nerve MCV and sural nerve SVC).

Vascular atherosclerotic changes are also considered a major effect of chronic carbon disulfide exposure. Several occupational studies have demonstrated an increase in the mortality from ischemic heart disease in CS_2 exposed workers (Hernberg *et al.*, 1970; MacMahon and Monson, 1988; Tiller, *et al.*, 1968; Tolonen *et al.*, 1979). A 2.5-fold excess in mortality from coronary heart disease in workers exposed to CS_2 was first reported by Tiller *et al.* (1968). A subsequent prospective study by Hernberg *et al.* (1970) found a 5.6-fold increased risk in coronary heart disease mortality and a 3-fold increased risk of a first nonfatal myocardial infarction in CS_2 exposed workers.

Egeland *et al.* (1992) and Vanhoorne *et al.* (1992) have reported that human exposure to CS_2 for more than one year causes increases in biochemical changes often associated with cardiovascular disease -- diastolic blood pressure, low density lipoprotein cholesterol, and apolipoproteins A1 and B. Egeland *et al.* (1992) used cross sectional data on 165 CS_2-exposed workers (245 controls) collected in 1979 by Fajen *et al.* (1981). The affected workers were exposed for at least 1 year in a viscose rayon factory to a estimated median TWA (8-hour) of 7.6 ppm. The Egeland *et al.* (1992) study indicated that modest CS_2 exposure (range 3.4 to 5.1 ppm, median 4.1 ppm) was associated with increased low density lipoprotein cholesterol (LDLc), the type of increase

associated with atherosclerotic heart disease. No significant differences were seen between controls and the low CS_2 exposed group (range 0.04 to 1.02 ppm, median 1.00 ppm). This study indicates a chronic NOAEL of 1.00 ppm and a LOAEL of 4.1 ppm for increased LDLc and diastolic blood pressure. Vanhoorne *et al.* (1992) identified increased LDL-cholesterol, apolipoprotein B, systolic and diastolic blood pressure indicative of a increased coronary risk in workers from a Belgium viscose rayon factory (115 exposed and 76 controls). CS_2 concentrations ranged from 1 to 36 ppm. Duration of exposure was not indicated. Even though these biochemical changes were observed, no significant increases in mild cardiovascular disease, such as angina, myocardial infarction, or ischemia were determined by ECG changes.

CS_2 causes reproductive toxicity in both males and females. Lancranjan *et al.* (1969), Cirla *et al.* (1978), and Wagar *et al.* (1981) studied male reproductive effects of occupational exposure to CS_2 and showed significant adverse effects on spermatogenesis, levels of serum FSH and LH, and libido, with these effects persisting in 66% of the workers subject to follow-up. Zhou *et al.* (1988) investigated pregnancy outcomes and menstrual disturbances in 265 women occupationally exposed to CS_2 and 291 controls. The CS_2-exposed women had significantly higher incidence of menstrual disturbances versus the control group (overall 34.9% vs. 18.2%). CS_2 levels varied between the five facilities, (exposure category means of low 3.1 mg/m^3, intermediate 6.5 mg/m^3 and high 14.8 mg/m^3), but all workers from these CS_2 facilities had significantly higher incidences of menstrual disturbance. Irregularity of menstruation was the most common disturbance, followed by abnormal bleeding. No evidence was observed to indicate an adverse effect on the term and outcome of pregnancy.

Unfortunately, the determination of possible LOAEL and/or NOAEL values for the major CS_2-related adverse effects from epidemiology studies, which predominately utilize workers from the viscose rayon industry, have often been limited by incomplete historical exposure measurements, concurrent exposure to other chemicals (including hydrogen sulfide or methylene chloride), lack of personal exposure determinations, and high variability of individual exposures due to decreases of plant CS_2 concentrations over time.

V. Effects of Animal Exposure

Studies investigating the potential for CS_2 toxicity in animals have usually been limited by intermediate or subchronic duration (less than 1 year) and a lack of multiple dose exposure groups. The neuropathologic changes consistently observed in rodents following CS_2 exposure include axonal swelling, demyelination, swelling at neuromuscular junctions, muscle atrophy and degeneration, damage to terminal axons, and nerve fiber breakdown (Clerici and Fechter, 1991; Colombi *et al.* 1981; Eskin *et al.*, 1988; Jirmanova and Lukas, 1984; Maroni *et al.*, 1979; Szendzikowski *et al.*, 1973). These adverse effects have been observed over a range of doses (250 to 800 ppm), but few studies have attempted to establish a dose response for this CS_2-induced neurotoxicity.

In a 90 day subchronic inhalation study, Sprague Dawley and Fisher 344 rats exposed discontinuously (6 hours/day, 5 days/week) to CS_2 developed morphological alterations in nerves

including axonal swelling and myelin degradation (Gottfried *et al.*, 1985). This study established a subchronic NOAEL of 50 ppm and a LOAEL of 300 ppm for morphological changes in nerves. A longer inhalation study in Wistar rats observed impairment in the conduction velocity of the sciatic and tibial nerves after 6 and 12 months of intermittent exposure to 289 ppm CS_2 (LOAEL of 289 ppm) (Knobloch *et al.*, 1979).

Wronska-Nofer (1973) showed a positive relationship between the level of triglycerides, the rate of cholesterol synthesis, and CS_2 exposure in Wistar rats exposed to 0, 73.8, 160, 321 or 546 ppm CS_2 for 5 hours/day, 6 days/week over 8 months. This study found a subchronic LOAEL of 73.8 ppm for disturbances in lipid metabolism (increase in serum cholesterol and serum triglycerides). Hepatic toxicity has also been induced in rats exposed to relatively high doses of CS_2, usually following pretreatment with liver inducers such as phenobarbital. Bond *et al.* (1969) showed that high doses of CS_2 to rats produced an increase in periportal liver fat, decreases in hepatic cytochrome P450 content and microsomal mixed function oxidase (MFO) activity. After phenobarbital induction, exposed rats produced more severe hepatoxicity characterized by hydropic degeneration and necrosis. Other hepatotoxic effects seen after CS_2 exposures greater than 400 ppm include increases in relative liver weight (Sokal, 1973), stimulation of liver microsomal lipid peroxidation (Wronska-Nofer *et al.*, 1986), and decreases in hepatic cholesterol synthesis (Simmons *et al.*, 1988).

VI. Derivation of U.S. EPA RfC

Study	Johnson *et al.* (1983); U.S. EPA (1995)
Study population	145 occupationally exposed workers and 212 nonexposed workers
Exposure method	Discontinuous occupational inhalation exposures (mean of 7.3 ppm and range of 0.6 to 16 ppm)
Critical effects	Reduction in motor nerve conduction velocities (decreased peroneal nerve MCV and sural nerve SVC)
LOAEL	7.3 ppm
NOAEL	Not observed
Average occupational exposure	2.6 ppm for LOAEL group
Benchmark concentration (BMC_{10})	17.7 ppm (continuity-weighted exposure of 6.3 ppm)
Exposure continuity	8 hr/day, 5 days/week
Human equivalent concentration	6.3 ppm for BMC
Exposure duration	Mean of 12.1 years (SD 6.9 years)
Subchronic factor	3
LOAEL factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	3
Modifying factor	3 (database deficiencies)
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.2 ppm (200 ppb; 0.7 mg/m^3; 700 $\mu g/m^3$)

The major strengths of the REL are the use of human data, the observation of a dose-response effect, and the duration of exposures. The major uncertainties are the poor quantitation of actual exposure magnitude over time and the limited nature of health effects studies conducted.

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CHRONIC TOXICITY SUMMARY

CARBON TETRACHLORIDE

(carbon chloride; carbon tet; freon 10; halon-104; methane tetrachloride; necatrine; tetrachlorocarbon; tetrachloromethane; tetraform; tetrasol; univerm)

CAS Registry Number: 56-23-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**40 μg/m^3**
Critical effect(s)	Increased liver weight and hepatic fatty infiltration in guinea pigs
Hazard index target(s)	Alimentary system; teratogenicity; nervous system

II. Physical and Chemical Properties

(HSDB, 1995)

Molecular formula	CCl_4
Molecular weight	153.8
Description	Colorless liquid
Specific gravity	1.59 at 20° C
Boiling point	76.7° C
Vapor pressure	91.3 mm Hg @ 20° C
Vapor density	5.3 at the boiling point (air = 1.0)
Solubility	Soluble in acetone, ethanol, benzene, carbon disulfide, slightly soluble in water
Conversion factor	1 ppm = 6.3 mg/m^3 @ 25° C

III. Major Uses or Sources

Carbon tetrachloride was formerly used for metal degreasing and as a dry-cleaning fluid, fabric-spotting fluid, fire-extinguisher fluid, grain fumigant and reaction medium (DeShon, 1979). Carbon tetrachloride is used as a solvent for the recovery of tin in tin-plating waste and in the manufacture of semiconductors. It is used in petrol additives, refrigerants, metal degreasing, and as a catalyst in the production of polymers. Carbon tetrachloride is also used as a chemical intermediate in the production of fluorocarbons and some pesticides (HSDB, 1995).

IV. Effects of Human Exposure

Kazantzis *et al.* (1960) evaluated 17 employees of a quartz processing factory who were occupationally exposed to 45-100 ppm (284-630 mg/m^3) carbon tetrachloride (CCl_4) vapor. Fifteen of the 17 workers complained of symptoms including nausea, anorexia, vomiting, flatulence, epigastric discomfort or distention, depressive symptoms, headache or giddiness for up to 4 months prior to the evaluation. A week after CCl_4 concentrations were reduced to 0-9 ppm with control measures, workers were symptom-free.

V. Effects of Animal Exposure

Adams *et al.* (1952) chronically exposed albino Wistar rats, guinea pigs, albino rabbits and rhesus monkeys to 0, 5, 10, 25, 50, 100, 200 and 400 ppm CCl_4 for varying durations. For each exposure group, two control groups were devised (unexposed and air-exposed controls) consisting of animals similar in age, sex, weight and number. The 2 control groups respnded similarly to the experimental protocol.

In the 100, 200 and 400 ppm exposure groups, mortality was excessive with moderate to severe liver cirrhosis and other various pathological changes in all the species tested. Fifteen male and 15 female rats were exposed to 50 ppm CCl_4 134 times for 187 days. They experienced decreased body weight gain and liver weight increase as well as moderate fatty degeneration and slight to moderate liver cirrhosis. Females showed kidney weight increase and four rats showed slight to moderate swelling of the kidney tubular epithelium. Guinea pigs (8 males and 8 females; 143 exposures in 200 days) showed depressed growth in the first two weeks, enlarged livers, moderate fatty degeneration and liver cirrhosis, and increased levels of liver total lipids, neutral fat, esterified cholesterol and plasma prothrombin clotting time.

The rabbit group of 2 males and 2 females which underwent 155 exposures to 50 ppm in 216 days showed slightly depressed growth and increased kidney weights, prolonged plasma prothrombin clotting time, moderate fatty degeneration and cirrhosis of the liver.

No change was seen in the group of 2 male monkeys exposed 198 times to 50 ppm in 277 days. One monkey experienced depressed weight gain as compared to the other monkey and the controls, but no other adverse effects were seen with respect to organ weights, tissue examination, total liver lipid, blood urea nitrogen, blood non-protein nitrogen, serum phosphatase, plasma prothrombin clotting time, phospholipid, neutral fat and free esterified cholesterol.

At 25 ppm CCl_4, 15 male and 15 female rats were exposed 137 times for 191 days. Early growth depression in males was observed, although final body weights did not significantly differ from the controls. Significant liver weight increase and slight to moderate fatty degeneration occurred. Liver lipid content was nearly twice the level of the controls and esterified cholesterol was five times that of the controls. For this exposure, phospholipid and neutral fat were not measured.

Five male guinea pigs were exposed 133 times over 185 days and 5 female guinea pigs were exposed 93 times over 126 days. Symptoms included growth depression, liver weight increase, increased plasma prothrombin clotting time, slight to moderate fatty degeneration, twice the level of the control total liver lipid and five times the control level of esterified cholesterol. After 178 exposures to 25 ppm over 248 days, rabbits (2 per sex) showed increased liver weights and slight to moderate liver cirrhosis and fatty degeneration.

Twenty male and 20 female rats were exposed 136 times over a period of 192 days to 10 ppm CCl_4. These rats exhibited increase in liver weight, slight to moderate fatty degeneration and total lipid, neutral fat and esterified cholesterol levels of twice the control levels. Guinea pigs (8 male and 8 female), who were exposed 139 times over 197 days, experienced liver weight increase, slight to moderate fatty degeneration without cirrhosis and increased levels of total lipid, neutral fat and esterified cholesterol. In an additional group of 18 male rats exposed 13 times to 10 ppm, slight fatty degeneration was seen as early as 17 days. Two male and two female rabbits tolerated the same regimen as the guinea pigs and showed no symptoms as a result of the exposure. Sixteen additional guinea pigs developed hepatic changes after 12 exposures in 16 days.

Twenty-five male and 23 female rats were exposed 145 times over 205 days to 5 ppm CCl_4 with no adverse effects seen. Nine male and nine female guinea pigs exposed 143 times over 203 days showed a statistically significant increase in the liver weights (females only), but only slightly higher liver lipid content. No additional histopathological effects were seen at this level of exposure.

Prendergast *et al.* (1967) exposed 15 Long-Evans or Sprague-Dawley rats, 15 guinea pigs, 3 rabbits, 2 dogs and 3 monkeys 30 times to a concentration of 515 $\pm$39 mg/m^3 (81.7 ppm) carbon tetrachloride (CCl_4) for 8 hours a day, 5 days a week, over a period of 6 weeks. Additionally, two 90 day continuous exposure studies were conducted. One study exposed 15 rats, 15 guinea pigs, 2 rabbits, 2 dogs and 3 monkeys to 61 $\pm$5.2 mg/m^3 CCl_4 and the other exposed 15 rats, 3 rabbits, 2 dogs and 3 monkeys continuously to 6.1 $\pm$0.3 mg/m^3 CCl_4 in inhalation chambers. Control groups consisted of 304 rats, 314 guinea pigs, 34 dogs, 48 rabbits and 57 monkeys. All the animals' weights were recorded prior to the study, at monthly intervals throughout the study, and at the conclusion of the study.

During the 6 week study, one monkey died following the 7th exposure, and 3 guinea pigs died following the 20th, 22nd and 30th exposures, respectively. Monkeys, guinea pigs, dogs and rabbits all exhibited weight loss. A high percentage of mottled livers was seen in all species except dogs. Histopathologic examination of the lungs and livers showed morphological changes in all the animals exposed to CCl_4 (most prominently the guinea pigs). The guinea pigs were the most sensitive species displaying discolored lungs, fatty livers, bile duct proliferation, fibrosis, focal inflammatory cell infiltration, hepatic cell degeneration and regeneration, early portal cirrhosis and alteration of lobular structure. Hepatic lipid content in the guinea pigs was 35.4 $\pm$10.7% compared to the control value of 11.0 $\pm$3.6%. Alterations of liver lipid content were also observed, to a lesser extent, in the other four species; the most severe alteration occured in the rats, less severe in rabbits and dogs, and the least severe in the monkeys.

During the 61 mg/m^3 (9.7 ppm) CCl_4 continuous exposure study, a total of 3 guinea pigs died after 47, 63 and 71 days. All of the monkeys were emaciated and experienced hair loss. Depressed body weight increases were seen in all of the animals when compared to the controls. Autopsies showed enlarged and/or discolored livers in a high percentage (not given) of monkeys, guinea pigs, rabbits and rats. Rats and guinea pigs showed hepatic fatty acid changes and a moderate reduction in succinic dehydrogenase activity was also evident in guinea pigs. Varying but lesser degrees of these changes were also seen in the other species tested.

The low concentration of 6.1 mg/m^3 (1 ppm) CCl_4 was attained by diluting the CCl_4 to 10% of the above concentration with *n*-octane, resulting in a solution of 6.1 mg/m^3 CCl_4 in 61 mg/m^3 of *n*-octane. The level of *n*-octane used was shown to be negligible by a *n*-octane control which yielded no effects (the current TLV is 1400 mg/m^3 (ACGIH, 1992)). No animals died during this study, and no signs of toxicity were noted. All exposed animals except the rats showed reduced weight gain when compared to the controls, and all species exhibited nonspecific inflammatory lung changes. Guinea pig liver lipid contents and serum urea nitrogen concentrations were similar to the control values. In several animals there were some nonspecific inflammatory changes in the liver, kidney and heart, but the authors did not attribute these to the chemical exposure. There was no other observed hematologic or histopathologic toxicity at this level.

Shimizu *et al.* (1973) exposed groups of 4 female Sprague-Dawley rats to 10, 50 and 100 ppm of CCl_4 vapor for 3 hours a day, 6 days a week for up to 6-8 weeks. The rats were sacrificed two days after the last inhalation. Accumulation of CCl_4 occurred in the adipose tissue and was measured after 1 and 3 weeks of exposure. For the 10 ppm group, accumulation was gradual, reaching a level of 1/3 the amount found in the 50 ppm group after 6 weeks. A slight increase of triglycerides in the liver (6.2-6.4 mg/g) was observed in the 10 ppm group, but no control group was used for comparison.

The intermittent exposure caused a more pronounced and higher number of change indices to occur (34 as opposed to the 17 change indices of the monotonous regimen), indicating a greater intensity of liver damage. Changes included a significant decrease in hippuric acid synthesis, presence of mitochondrial enzymes (glutamate dehydrogenase and ornithine carbonyl transferase) in the blood, indicating severe damage to hepatocytes, significant increase in cytoplasmic enzyme activity and a decrease in the level of cytochrome p-50 in liver tissue. The effects seen in the monotonous group were the same variety as those in the intermittent group, but were less intense. The content of CCl_4 in the blood was similar for both the intermittent and monotonous exposure groups. Another test was performed over a period of 27 days varying the regimen, and therefore the concentration, of intermittent exposure while keeping the TWA level of CCl_4 stable. Increasing the concentration threefold or fivefold with five 10 minute peaks did not potentiate the toxic effects. Varying the regimen tenfold to five 5-minute peaks (peak exposure 402 mg/m^3, 63.8 ppm) with a time weighted average exposure of 6.5 ppm (41$\pm$1 mg/m^3) did however, result in more severe liver damage.

Sakata *et al.* (1987) exposed 10-15 male Sprague-Dawley rats to <10 ppm CCl_4 vapor for 15 minutes a day, twice a week for 8 weeks. All the rats experienced chronic liver damage involving nodular liver surfaces and extensive fibrosis. Researchers also found similar results in rats after 8 weeks of subcutaneous injections of 0.1 mL of 50% CCl_4 solution in olive oil twice a week.

Ideura *et al.* (1993) exposed male Wistar rats to CCl_4 vapor for 7 minutes, 3 times a week for 6-10 weeks (concentration unspecified). Six experimental groups of 4-5 rats were used, two of which were exposed for 10 weeks, another two for 6 weeks, and two unexposed control groups. Following the last exposures, rats were injected with varying amounts of endotoxin (1.0 mL lipopolysaccharide LPS). The rats were sacrificed 24 hours after the injection and processed for histological examination. The rats' left kidneys and livers were examined to reveal liver cirrhosis with destruction of normal structure and massive ascites retention after 10 weeks of exposure as compared to the controls. Those exposed for 6 weeks exhibited an increase in fibrous tissue. The control groups displayed normal liver structure. Researchers found that rats previously resistant to endotoxin became susceptible following CCl_4 exposure manifested as induced acute renal tubular necrosis in cirrhotic rats.

Yoshimura *et al.* (1993) performed a similar experiment to that of Ideura *et al.* (1992) by exposing male Wistar rats for 6 (5 rats) and 10 weeks (5 rats) to 99% CCl_4 vapor for 3 minutes a day. A control group of 5 rats was given phenobarbitone for 10 weeks. After 24 hours following the final exposure, rats were injected with endotoxin. Six weeks of CCl_4 exposure caused liver fibrosis with bridging fibrosis, while 10 weeks of exposure to CCl_4 caused liver cirrhosis and destruction of the normal liver architecture.

Pregnant rats were exposed to 0, 300, or 1000 ppm (0, 1938, or 6460 mg/m^3) carbon tetrachloride for 7 hours/day on days 6-15 of gestation. Significant fetal growth retardation, measured by decreased crown-rump length and body weight, was observed in the offspring of the exposed groups (n = 22 litters) compared with controls (n = 43 litters). Subcutaneous edema was observed in the 300 ppm group but not in the 1000 ppm group. Sternebral anomalies were observed in the 1000 ppm group.

Table 1. Effects of Chronic CCl_4 Exposure (Adams *et al.*, 1952)

Species	**Concentration (ppm)**	**Group size**	**Endpoint**	**Exposure scenario** (days exposed/ experiment length)
rats (male)	50 ppm	15	liver damage: fatty degeneration and cirrhosis; growth depression	134/187
rats (female)	50 ppm	15	same effects as males with the addition of increased kidney weight	134/187
guinea pigs	50 ppm	16	liver damage: fatty degeneration and cirrhosis; growth depression	143/200
rabbits	50 ppm	4	enlarged kidney; liver damage: fatty degeneration and cirrhosis; growth depression	155/216
monkeys	50 ppm	2	one experienced growth depression	198/277
rats	25 ppm	30	liver damage; early growth depression	137/191
guinea pigs (male)	25 ppm	5	liver damage: fatty degeneration; growth depression	133/185
guinea pigs (female)	25 ppm	5	liver damage: fatty degeneration; growth depression	93/126
rabbits	25 ppm	4	liver damage: fatty degeneration; and cirrhosis	178/248
rats	10 ppm	40	liver damage: fatty degeneration	136/192
guinea pigs	10 ppm	16	liver damage: fatty degeneration	139/197
rats	5 ppm	48	no adverse effects	145/205
guinea pigs (male)	5 ppm	9	no adverse effects	143/203
guinea pigs (female)	5 ppm	9	liver damage	143/203

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Adams *et al.* (1952)
Study population	9 male and 9 female guinea pigs
Exposure method	Discontinuous whole-body inhalation
Critical effects	Increase in liver weight and liver lipid content in females
LOAEL	5 ppm
NOAEL	Not observed
Exposure continuity	7 hours/day, 5 days/week
Average experimental exposure	1.0 ppm
Human equivalent concentration	1.7 ppm (gas with systemic effects, based on RGDR = 1.7 for lambda (a) : lambda (h) (Gargas *et al.* 1989))
Exposure duration	143 exposures over 203 days (7.3 months)
LOAEL uncertainty factor	10
Subchronic factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300

Inhalation reference exposure level 0.006 ppm; 6 ppb (40 μg/m^3; 0.04 mg/m^3)

Of the 2 adequate chronic inhalation studies available on CCl_4, the Adams *et al.* (1952) study was chosen over the Prendergast *et al.* (1967) study as the key reference for the carbon tetrachloride chronic REL. The Adams *et al.* (1952) experiment was conducted over a longer duration. In comparison, the Prendergast study was only conducted for a subchronic period of 6 weeks. In addition, the Adams study contained more specific endpoints of liver damage that were consistent with the mechanism of carbon tetrachloride toxicity. Both studies resulted in hepatic effects with exposed rats appearing less sensitive than the affected monkeys or guinea pigs.

The major strength of the REL is the use of a chronic exposure study. The major uncertainties are the lack of human data, the lack of a NOAEL observation, the small sample sizes used, and the lack of comprehensive multiple dose studies. For comparison, conversion of the oral U.S. EPA RfC value of 0.7 μg/kg/day to an equivalent inhalation value results in a concentration of 2.5 μg/m^3.

VII. References

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CHRONIC TOXICITY SUMMARY

CHLORINATED DIBENZO-P-DIOXINS AND CHLORINATED DIBENZOFURANS

(INCLUDING 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN)

(Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) including 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) which is the principal congener of concern based on toxicity)

CAS Registry Number: 1746-01-6 (TCDD); 51207-31-9 (TCDF)

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.00004 µg/m^3 (40 pg/m^3)**
Oral reference exposure level	**1 x 10^{-8} mg/kg/day (10 pg/kg/day)**
Critical effect(s)	Increased mortality, decreased weight gain, depression of erythroid parameters, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues in rats.
Hazard index target(s)	Alimentary system; immune system; reproductive system; teratogenicity; endocrine system; respiratory system; circulatory system

II. Physical and Chemical Properties (HSDB, 1995)

Molecular Formula	$C_{12}H_4Cl_4O_2$ (TCDD)
Molecular Weight	321.97 g/mol (TCDD)
Description	white crystalline powder at 25° C
Specific Gravity	1.827 g/ml (estimated)
Boiling Point	412.2°C (estimated)
Vapor Pressure	1.52 x 10-9 mm Hg at 25°C
Solubility	In water: 7.91 ng/L at 20-22°C; 19.3 ng/L at 22°C
Log Kow	6.15-7.28
Log Koc	6.0-7.39
Henry's Law Constant	8.1 x 10^{-5} ATM-m^3/mol

III. Major Uses and Sources

The chlorinated dioxins and furans are generated as by-products from various combustion and chemical processes. PCDDs are produced during incomplete combustion of chlorine containing wastes like municipal solid waste, sewage sludge, and hospital and hazardous wastes. Various metallurgical processes involving heat, and burning of coal, wood, petroleum products and used tires for energy generation also generate PCDDs. Chemical manufacturing of chlorinated phenols (e.g., pentachlorophenol), polychlorinated biphenyls (PCBs), the phenoxy herbicides (e.g., 2,4,5 T), chlorinated benzenes, chlorinated aliphatic compounds, chlorinated catalysts and halogenated diphenyl ethers are known to generate PCDDs as a by-product under certain conditions. While manufacture of many of these compounds and formulations has been discontinued in the United States, continued manufacture elsewhere in the world combined with use and disposal of products containing PCDD by-products results in the inadvertent release of PCDDs into the environment. Industrial and municipal processes in which naturally occurring phenolic compounds are chlorinated can produce PCDDs, with the best example being chlorine bleaching of wood pulp in the manufacture of paper products. Additionally, municipal sewage sludge has been documented to occasionally contain PCDDs and PCDFs.

IIIa. 2,3,7,8 Tetrachlorodibenzo-p-dioxin Toxic Equivalents

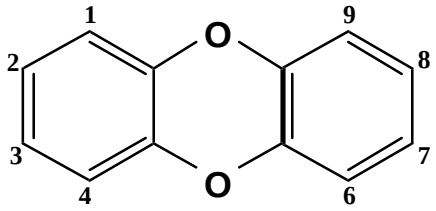
2,3,7,8-Tetrachlorodibenzo-p-dioxin is considered the most potent congener of the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) families of compounds. Potency of PCDD and PCDF congeners correlates with the binding affinity to the cytosolic Ah receptor. Structure activity studies have demonstrated that optimal biological activity and Ah-receptor binding requires congeners with a planar conformation and chlorines at the corners of the molecule at the 2,3,7,8 positions (Poland and Knutson, 1982; Safe, 1986). Chlorines at both ortho positions in these molecules (i.e. positions 1 and 9) sterically hinder a planar conformation which lessens the congeners' biological activity. Thus only 15 of 210 different PCDDs and PCDFs congeners possess significant biological activity based on chlorines in the 2,3,7,8 positions and some degree of planar conformation (Safe, 1986; U.S. EPA 1989). These include two tetrachloro-congeners; 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8- tetrachlorodibenezofuran, three pentachloro congeners; 1,2,3,7,8-pentachlorodibenzo-p-dioxin, 1,2,3,7,8-pentachlorodibenzofuran, and 2,3,4,7,8-pentachlorodibenezofuran, seven hexachloro congeners; 1,2,3,4,7,8 or 1,2,3,6,7,8 or 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins and hexachlorodibenzofurans and 2,3,4,6,7,8-hexachlorodibenezofuran, and three heptachloro congeners; 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin, 1,2,3,4,6,7,8-heptachlorodibenezofuran and 1,2,3,4,7,8,9-heptachlorodibenezofuran (U.S. EPA, 1989). The structures of the dibenzo-p-dioxins and dibenzofurans along with their numbering schemes are shown in Figure 1. Toxic equivalents are calculated relative to the most potent congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and determined based on structure activity studies examining relative affinity for the Ah receptor as well as relative toxicity of different congeners. Values for the international system of toxic equivalents are provided in Table 1 (U.S. EPA, 1989). The use of toxic equivalent factors in the state of California will be presented in a companion document.

Table 1. International Toxic Equivalency Factors (I-TEFs) for PCDDs and PCDFs Chlorinated in the 2,3,7, and 8 Positions. (U.S. EPA 1989.)

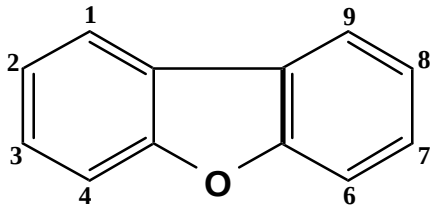
Compound[1,2]	I-TEF
Mono-, Di-, and Tri-CDDs and CDFs	0
TetraCDD	
2,3,7,8-substituted	1.0
Others	0
PentaCDD	
2,3,7,8-substituted	0.5
Others	0
HexaCDD	
2,3,7,8-substituted	0.1
Others	0
HeptaCDD	
2,3,7,8-substituted	0.01
Others	0
OctaCDD	0.001
TetraCDF	
2,3,7,8	0.1
Others	0
PentaCDF	
1,2,3,7,8-PentaCDF	0.05
2,3,4,7,8-PentaCDF	0.5
others	0
HexaCDF	
2,3,7,8-substituted	0.1
Others	0
HeptaCDF	
2,3,7,8-substituted	0.01
Others	0
OctaCDF	0.001

1CDD designates chlorinated dibenzo-p-dioxin
2CDF designates chlorinated dibenzofuran

Figure 1. Structures of the Dibenzo-p-dioxins and Dibenzofurans-



Dibenzo-p-dioxin



Dibenzofuran

IV. Effects of Human Exposure

The information available on possible chronic toxic effects in humans is complicated by the relative insensitivity of epidemiological studies, the limited ability of case studies of exposed individuals to establish cause and effect relationships, the heterogeneous nature of human populations, the broad spectrum of exposures to other toxic agents in the human environment, and the episodic exposure of many of the exposed human populations which have been studied (e.g., Seveso, Italy. As a result, a limited number of effects have been associated with exposure to dioxins in humans. The meaning of these effects in terms of toxicity in most cases remains to be clarified. The majority of information comes from cross-sectional medical studies. Chloracne is the most widely recognized effect of exposure to 2,3,7,8-TCDD and TCDD-like PCDDs and PCDFs. Chloracne is a persistent condition which is characterized by comedones, keratin cysts and inflamed papules and is seen after acute and chronic exposure to various chlorinated aromatic compounds (Moses and Prioleau, 1985). Other dermal effects include hyperpigmentation and hirsutism or hypertrichosis (Jirasek *et al.*, 1974; Goldman 1972; Suskind *et al.*, 1953; Ashe and Suskind, 1950), both of which appear to resolve themselves more quickly over time than chloracne, making them more of an acute response rather than a chronic response (U.S. EPA, 1994a). Epidemiological data available for 2,3,7,8-TCDD have not allowed a determination of the threshold dose required for production of chloracne (U.S. EPA, 1994b). Case studies suggest that there may be a relationship between 2,3,7,8-TCDD exposure and hepatomegaly (Reggiani, 1980; Jirasek *et al.*, 1974; Suskind *et al.*, 1953; Ashe and Suskind, 1950) and hepatic enzyme changes (Mocarelli *et al.*, 1986; May, 1982; Martin 1984; Moses *et al.*, 1984); nevertheless, cross sectional epidemiological studies of trichlorophenol (TCP) production workers (Suskind and Hertzberg., 1984; Bond *et al.*, 1983; Moses *et al.*, 1984; Calvert *et al.* 1992), Vietnam veterans (Centers for Disease Control Vietnam Experience Study,

1988; Roegner *et al.*, 1991) and Missouri residents (Webb, 1989; Hoffman *et al.*, 1986) found little evidence for an association between exposure and hepatomegaly suggesting that this is not a chronic response. There is a consistent pattern of increased levels of serum gamma glutamyl transferase in populations exposed to 2,3,7,8-TCDD which is presumably of hepatic origin (Mocarelli, 1986; Caramaschi *et al.*, 1981, May, 1982; Martin, 1984; Moses *et al.*, 1984; Calvert *et al.*, 1992; Centers For Disease Control Vietnam Experience Study, 1988). Two cross sectional studies have associated diabetes and elevated fasting serum glucose levels with relatively high serum 2,3,7,8-TCDD levels (Sweeney *et al.*, 1992; Roegner *et al.*, 1991) however other studies provided mixed results (Moses *et al.*, 1984; Centers for Disease Control Vietnam Experience Study 1988; Ott *et al.*, 1993). TCDD has been associated with effects on reproductive hormonal status in males. The likelihood of abnormally low testosterone levels was 2 to 4 times greater in individuals with serum 2,3,7,8-TCDD levels above 20 pg/ml (Egeland *et al.* 1994) and increased serum levels of luteinizing hormone and follicle stimulating hormone have been documented (Egeland *et al.* 1994). A number of other effects have been reported that were either not seen as chronic effects or effects seen long term in only one population of exposed persons. These include elevated liver enzymes (aspartate aminotransferase and alanine aminotransferase), pulmonary disorders, neurologic disorders, and changes in porphyrin metabolism and kidney disorders (U.S. EPA, 1994c). Areas in which there is presently insufficient information to draw solid conclusions include effects on the circulatory system, reproductive effects, immunological effects, effects on metabolism and handling of lipids, and on thyroid function (U.S. EPA, 1994c). Recent findings in Rhesus monkeys have shown 2,3,7,8-TCDD to cause endometriosis (Reier *et al.*, 1993) and currently epidemiological studies are underway to determine if there is an association between TCDD exposure and endometriosis in human populations exposed by the Seveso accident.

Potential effects of a toxicant on normal fetal development include fetal death, growth retardation, structural malformations and organ system dysfunction. Evidence for all four of these responses has been seen in human populations exposed to dioxin-like compounds. In these poisoning episodes populations were exposed to a complex mixture of halogenated aromatic hydrocarbons contained within PCBs, PCDFs and PCQs mixtures thus limiting the conclusions that could be drawn from the data. In the Yusho and Yu-Cheng poisoning episodes, human populations consumed rice oil contaminated with PCBs, PCDFs and PCQs. Yu-Cheng women experienced high perinatal mortality in hyperpigmented infants born to affected mothers (Hsu *et al.* 1985). This occurred in women with overt signs of toxicity (chloracne) (Rogan, 1982) and Rogan notes that it appears that when there is no sign of toxicity in the mother the likelihood of fetotoxicity appears to lessen considerably in the infants. Signs of toxicity from dioxin like compounds were absent in infants born to mothers apparently not affected in the Seveso, Italy and Times Beach, Missouri, incidents (Reggiani, 1989; Hoffman and Stehr-Green, 1989) supporting this conclusion. There was an increased incidence of decreased birth weight in infants born to affected mothers in the Yusho and Yu-Cheng incidents suggesting fetal growth retardation (Wong and Huang, 1981; Law *et al.*, 1981; Lan *et al.*, 1989; Rogan *et al.*, 1988). The structural malformation, rocker bottom heel was observed in Yusho infants (Yamashita and Hayashi, 1985) making this malformation a possible result of exposure to dioxin like compounds, nevertheless, it is unknown if these compounds produce malformations in humans. Evidence for possible organ system dysfunction in humans comes from a study of Yu-Cheng

children which found that children exposed in utero experienced delays in attaining developmental milestones, and exhibited neurobehavioral abnormalities (Rogan *et al.* 1988) suggesting involvment of CNS function. Dysfunction of dermal tissues are noted in exposed infants of the Yusho and Yu-Cheng incidents and characterized by hyperpigmentation of the skin, fingernails, and toenails, hypersecretion of the meibomian glands, and premature tooth eruption (Taki *et al.*, 1969; Yamaguchi *et al.*, 1971; Funatsu *et al.*, 1971; Wong and Huang, 1981; Hsu *et al.*, 1985; Yamashita and Hayashi, 1985; Rogan *et al.*, 1988; Rogan, 1989; Lan *et al.*, 1989).

V. Effects of Animal Exposure

The toxicity to laboratory animals encompasses a number of areas including changes in energy metabolism manifested as wasting syndrome, hepatotoxicity, effects on tissue of epithelial origin, various endocrine effects, effects on vitamin A storage and use, immune system effects and reproductive and developmental toxicity. The limited number of chronic studies available do not examine all these endpoints. Therefore subchronic exposures are included here in order to provide a more complete coverage of potential chronic toxic effects of these compounds.

Wasting syndrome is one of the most broadly occurring toxic effects. The wasting syndrome is characterized by loss of adipose tissue and lean muscle mass and is produced in all species and strains tested, but there are difference in sensitivity (U.S. EPA 1994d; Peterson *et al.*, 1984; Max and Silbergeld, 1987). Numerous studies have not yet established the mechanism of wasting syndrome (U.S. EPA 1994e). Hepatotoxicity is also seen in all species tested with considerable variation in species sensitivity (U.S. EPA, 1994d). TCDD induces hyperplasia and hypertrophy of liver parenchymal cells. Morphological and biochemical changes in the liver include increased SGOT and SGPT, induction of microsomal monooxygenases and proliferation of the smooth endoplasmic reticulum, porphyria, increased regenerative DNA synthesis, hyperlipidemia, hyperbilirubinemia, hyperchloesterolemia, hyperproteinemia, degenerative and necrotic changes, mononuclear cell infiltration, multinucleated giant hepatocytes, increased numbers of mitotic figures, and parenchymal cell necrosis (U.S. EPA 1994d; WHO/IPCS, 1989). Epithelial effects seen include chloracne (rabbit ear and the hairless mouse) (Jones and Krizek, 1962; Schwetz *et al.*, 1973) and hyperplasia and/or metaplasia of gastric mucosa, intestinal mucosa, the urinary tract, the bile duct and the gall bladder (U.S. EPA 1994f). TCDD exposure results in endocrine like effects including epidermal growth factor like effects such as early eye opening and incisor eruption in the mouse neonate (Madhukar *et al.*, 1984), glucorticoid like effects such as involution of lymphoid tissues (U.S. EPA 1994g; Sunahara *et al.*, 1989), alteration in thyroid hormone levels and in some cases thyroid hormone like effects (WHO/IPCS, 1989; Rozman *et al.*, 1984), decreases in serum testosterone and dihydrotesterone (Mittler *et al.*, 1984; Keys *et al.*, 1985; Moore *et al.*, 1985), and changes in arachidonic acid metabolism and prostaglandin synthesis (Quilley and Rifkind, 1986; Rifkind *et al.*, 1990). TCDD is known to decrease hepatic vitamin A storage (Thunberg *et al.*, 1979). TCDD and other dioxin like PCDDs and PCDFs are potent suppressers of both cellular and humoral immune system function, characteristically producing thymic involution at low doses and involution of other lymphoid tissues at higher doses (U.S. EPA 1994h).

In animal studies there is a large body of information available documenting both developmental and reproductive toxicity of 2,3,7,8-TCDD and other PCDDs and PCDFs. These compounds are acutely toxic to early life stages of fish and birds with fish being most sensitive (LD_{50} of 0.4 µg/kg for rainbow trout sac fry eggs, LD_{50} of 34 ng/kg for lake trout eggs) and some species of birds are also relatively sensitive (LD_{50} of 0.25 µg/kg for chicken eggs) (Peterson *et al.*, 1993). 2,3,7,8-TCDD has been documented to increase the incidence of prenatal mortality in a number of species of laboratory animals including the Rhesus monkey, Guinea pig, rabbit, rat, hamster, and mouse (Peterson *et al.*, 1993). Exposure to 2,3,7,8-TCDD during gestation produces a characteristic set of fetotoxic responses in most laboratory animals which includes: thymic hypoplasia, subcutaneous edema, and decreased growth (Peterson *et al.*, 1993). More species specific responses include cleft palate formation in the mouse at doses below maternal toxicity (More *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990), intestinal hemorrhage in the rat. (Sparschu *et al.*, 1971), hydronephrosis in the mouse and hamster (Moore *et al.*, 1973; Smith *et al.*, 1976; Couture *et al.*, 1990; Birnbaum *et al.*, 1989; Olson *et al.*, 1990), and extra ribs in the rabbit (Giavini *et al.*, 1982). Female rats have also been found to be affected by perinatal exposure to 2,3,7,8-TCDD with clefting of the clitoris, incomplete or absent vaginal opening and a smaller vaginal orifice after a dose of 1 µg/kg to the mother on day 15 of gestation (Gray *et al.*, 1993).

A number of effects on adult reproductive function are seen in male animals exposed in utero to 2,3,7,8-TCDD. TCDD reduces plasma androgen levels in the adult male rat and perinatal exposure decreases spermatogenesis, spermatogenic function and reproductive capability, feminizes male sexual behavior, and feminizes male gonadotrophic function (LH secretion) (Mably *et al.*, 1991; Mably *et al.*, 1992a,b,c). Evidence suggests that these effects are the result of impaired sexual differentiation of the CNS which in male rats is dependent on exposure of the developing brain to testosterone.

There are numerous studies detailing the effects of the PCDDs, PCDFs and other dioxin like compounds, however a large number of these studies were conducted as either acute or subchronic exposures (studies in which it is unlikely that body burdens had reached steady state levels). Detailed below are three chronic studies which were considered in the setting of a chronic toxicity standard.

The most definitive study of chronic toxicity in rats is that of Kociba *et al.* (1978). This study involved the administration of 2,3,7,8-TCDD in the diet at doses of 1 ng/kg/day, 10 ng/kg/day, and 100 ng/kg/day to groups of 50 male and 50 female Sprague Dawley rats for two years. A group of 86 male and 86 female rats receiving diet with solvent vehicle alone served as controls. The following observations were made excluding carcinogenic effects which were seen at the 100 ng/kg/day dose. The 100 ng/kg/day dose increased mortality, decreased weight gain, depressed erythroid values, increased urinary excretion of porphyrins and delta-aminolevulinic acid, and increased serum activities of alkaline phosphatase, gamma-glutamyl transferase, and glutamic-pyruvic transaminase. Histopathologic changes were noted in the liver, lymphoid tissue, respiratory and vascular tissues. The primary ultrastructural change in the liver was

proliferation of the rough endoplasmic reticulum. At the 10 ng/kg/day dose the severity of toxic symptoms was less than that of the 100 ng/kg/day dose and included increased urinary excretion of porphyrins in females as well as liver and lung lesions. The 1 ng/kg/day dose produced no discernible significant toxic effects. Interpretation of this study by the authors was that the 1 ng/kg/day dose was a NOAEL.

Two chronic toxicity studies are available in the mouse. The first is a one year study conducted by Toth *et al.* (1979) using male Swiss Mice administered weekly oral doses of 7, 700, and 7000 ng/kg/day. In this study 2,3,7,8-TCDD administration resulted in amyloidosis and dermatitis in 0 of 38 control animals, 5 of 44 animals receiving 7 ng/kg/day, 10 of 44 animals receiving 700 ng/kg/day and 17 of 43 animals receiving 7,000 ng/kg/day. The other study was from the NTP 1982 gavage study (NTP, 1982) in B6C3F1 mice. This study employed groups of 50 male and 50 female mice. The males received doses of 0, 10, 50, and 500 ng/kg/week by gavage for two years while female mice received doses of 0, 40, 200, and 2000 ng/kg week by gavage for two years. No adverse effects were seen at the lowest doses tested in each sex which corresponds to a NOAEL of approximately 1.4 and 6 ng/kg/day for males and females respectively. Neither chronic toxicity study in mice reported data on enzyme activity.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Kociba *et al.* (1978)
Study population	Sprague-Dawley Rats of both sexes (50/treatment group/sex)
Exposure method	Continuous dietary exposure starting at seven weeks of age for 2 years
Critical Effects	Increased mortality, decreased weight gain, depression of hematologic measures, increased urinary excretion of porphyrins and delta-aminolevulinic acid, increased serum activities of alkaline phosphatase, gamma-glutamyl transferase and glutamic-pyruvic transaminase, gross and histopathological changes in the liver, lymphoid tissue, lung and vascular tissues
Observed LOAEL	210 ppt in diet (0.01 µg/kg/day)
Observed NOAEL	22 ppt in diet (0.001 µg/kg/day)
Exposure continuity	Continuous exposure via the diet
Exposure duration	2 years
Subchronic uncertainty factor	1
LOAEL uncertainty factor	1
Interspecies uncertainty factor	10
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Oral reference exposure level	10 pg/kg/day

Inhalation reference exposure level 40 pg/m^3 (0.00004 µg/m^3)

The data available for chronic toxic effects in humans has a number of limitations: some studies did not determine the body burden of compounds necessary to estimate dose, the Yusho and Yu-Cheng poisoning episodes have uncertainty because exposure was to complex mixtures of halogenated aromatic hydrocarbons rather than individual congeners, and epidemiological studies and case studies have limitations in determining cause and effect relationships. Therefore an animal study was chosen for determination of a NOAEL/LOAEL. The study chosen for use was that of Kociba *et al.* (1978) based on the duration of the study (2 years), the number of animals employed (50 per treatment group per sex), testing of both sexes, a dose range which spanned from an apparent NOAEL to severe hepatic effects including carcinogenic effects, a complete histopathological examination of all organ systems, examination of urinary excretion of porphyrins and delta-aminolevulinic acid, and determination of serum activities of alkaline phosphatase, gamma-glutamyl transferase, and glutamic-pyruvic transaminase. The elevation of human serum values for gamma-glutamyl transferase is one of the consistently seen chronic responses in exposed human populations and reflects changes in liver biochemistry. Thus the examination of markers of liver toxicity also altered in animal models of chronic toxicity make the Kociba study an appropriate choice for detecting potential chronic toxic effects of 2,3,7,8-TCDD in humans. The NOAEL in the Kociba *et al.* (1978) study was determined to be 1 ng/kg body weight/day. For the purposes of determining the REL the 1 ng/kg/day dose was considered to be a NOAEL based upon the observations of Kociba *et al.* (1978).

NOAEL's from a number of other studies compare favorably with the 1 ng/kg/day NOAEL including the NTP (1982) study in B6C3F1 mice, and the NOEL for enzyme induction in rats and marmosets calculated by Neubert (1991) of 1 ng/kg. Furthermore the 1 ng/kg/day NOAEL is lower than the LOAEL's observed by Toth *et al.* (1979) of 7 ng/kg/day in mice and observed by Schantz *et al.* (1978) of 2.3 ng/kg/day in rhesus monkeys. Current exposure assessments for 2,3,7,8-TCDD and other dioxin-like compounds including the PCBs, PCDDs, and PCDFs estimate that the average daily background dose in the U.S. is 3-6 pg TEQ/kg/day (U.S. EPA 1994i) also placing the REL close to background exposures. The REL of 10 pg/kg/day should be protective of chronic effects on liver function and avoid significant increases in exposure over the background level of human exposure.

The strengths of the inhalation REL include the availability of chronic exposure data from a well-conducted study with histopathological analysis, the observation of a NOAEL, and the demonstration of a dose-response relationship. Major areas of uncertainty are the lack of adequate human exposure data and the lack of chronic inhalation exposure studies.

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CHRONIC TOXICITY SUMMARY

CHLORINE

CAS Registry Number: 7782-50-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.06 µg/m^3**
Critical effect(s)	Hyperplasia in respiratory epithelium in female rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995 except as noted)

Molecular formula	Cl_2
Molecular weight	70.906 (Weast, 1989)
Description	Yellow/green gas
Specific gravity	2.5 (air=1)
Boiling point	-34.6° C
Vapor pressure	5 atm @ 10.3° C
Solubility	Slightly soluble in water (310 mL per 100 mL water at 10° C; 1.46 g per 100 mL water at 0 degrees C)
Conversion factor	1 ppm = 2.9 mg/m^3 @ 25° C

III. Major Uses and Sources

Chlorine is commonly used as a household cleaner and disinfectant (HSDB, 1995). In an industrial setting, chlorine is widely used as an oxidizing agent in water treatment and chemical processes. Chlorine is an integral part of the bleaching process of wood pulp in pulpmills.

IV. Effects of Human Exposure

Shi and associates (1990) evaluated 353 workers from a diaphragm cell chlorine chemical plant. The workers ranged in age from 23-52 years with an average of 42.4 years. Two groups were compiled with respect to the workers' length of exposure in years. Group A consisted of 220 workers who were employed/ exposed for 10-25 years. Group B consisted of 133 workers employed for less than 10 years. Both groups of workers were exposed to a range of 2.60-11.0 mg/m^3 (0.37-1.75 ppm) chlorine. The control group's average age was 39.7 years (ranging from 26-55 years), and it consisted of 192 workers not exposed to chlorine, but working within the same plant. For all the groups, respiratory symptoms and smoking habits were evaluated as

well as clinical examinations, ENT examinations, chest x-rays and pulmonary function tests. Groups A and B showed 3-8 times higher incidence of upper airway complaints than the control workers. Current smokers in groups A and B experienced the highest incidence of pulmonary symptoms and group A workers had a higher prevalence of rhino-pharyngeal signs than the control workers. Abnormalities in chest x-rays were seen in 8.6% of group A workers and in 2.8% of group B workers, compared to 2.3% of the control workers. Groups A and B showed significantly impaired pulmonary function in tests V50/H and FEF25-75 compared with the control group, and group A showed reduced FEV1 results compared to the control group.

Kennedy *et al.* (1991) compared 321 pulpmill workers (189 of whom were exposed to chlorine or chlorine dioxide "gassings") to a control group of 237 rail yard workers in similar working conditions but not exposed to chlorine (79% and 84% respective participation rate). The workers had been employed for an average of 13 years at the pulpmill and 12.7 years at the rail yard. Chlorine gas and chlorine dioxide levels were measured together over a 4 week period during mainly a 12 hour shift. Time weighted averages (TWA) were <0.1 ppm, with the highest of <0.1-0.3 ppm. A significantly higher prevalence of wheezing was seen in pulpmill workers (both smokers and nonsmokers) who had reported more than one episode of chlorine "gassing" as compared to the rail yard workers and pulpmill workers with no chlorine gas exposure. More airflow obstruction was observed in exposed workers in spite of their nonsmoking and ex-smoking status, correlating to significantly lower average values for MMF and the FEV_1 to FVC ratio. Comparison of pulpmill workers exposed to chlorine and /or chlorine dioxide with those pulpmill workers not exposed, suggests that chronic respiratory health impairment is associated with exposure to chlorine and/or chlorine dioxide. These researchers hypothesized that after the first high exposure incident, an inflammatory response occurred in small airways and that this reaction did not resolve in those workers who were continuously or repeatedly exposed to the irritant. It was also suggested that chronic airflow obstruction caused by repeated minor exposures led to chronic respiratory disability in some of the workers.

Patil *et al.* (1970) evaluated the exposure of 332 male diaphragm cell workers to 0.006-1.42 ppm chlorine gas (a range with a time-weighted average of 0.146 $\pm$0.287; most workers were exposed to less than 1 ppm). A control group consisting of 382 workers from 25 representative chlorine manufacturing plants was also studied. Both groups were comprised of men between the ages of 19-69 with a mean age of 31.2 $\pm$11.0 years. Physical examinations (blood and urine analysis, chest x-rays and electrocardiograms) were conducted, in most cases, within the first six months of the study year. At two month intervals, each plant was surveyed and chlorine levels were determined. Exposed employees were grouped according to job classification. Researchers found the average number of exposure years for the study group to be 10.9 $\pm$ 2.8 years and concluded that the exposure level had no correlation to the number of years exposure. Ninety-eight of the 332 workers were found to have abnormal teeth and gums, but no dose-response relationship was concluded. Similarly, no dose-response relationships were shown with the symptoms of sputum production, cough, dyspnea, history of frequent colds, palpitation, chest pain, vital capacity, maximum breathing capacity and forced expiratory volume. Any deterioration in pulmonary function was shown to be age related. Of the 332 exposed workers, 9.4% experienced abnormal EKG's. 8.5% of the control group showed the same abnormalities, but this difference was not significant. Above 0.5 ppm, an increase appeared in the incidence of

fatigue. No neurological defects developed and there was no noted prolonged anoxia as a result of the chlorine exposure. Also, no consistent gastrointestinal trouble or abnormal incidence of dermatitis was found. Exposed workers showed elevated white blood cell counts and decreased hematocrit values compared to the control group.

Chang-Yeung *et al.* (1994) conducted a clinical, functional and pathological study of three pulpmill workers who, after years of intermittent exposure to pulpmill "gassings," developed a cough, wheeze (chest tightness) and shortness of breath. The subjects were evaluated on the basis of lung function tests and nonspecific bronchial hyperresponsiveness. Previous "gassing" episodes caused immediate symptoms in the subjects, but did not cause persistent respiratory symptoms. However, the subjects were admitted for emergency hospital treatment after a severe exposure. Following that episode, the subjects were diagnosed with irritant-induced asthma and treated with steroid therapy. Changes were seen in the subjects' bronchial mucosa which were similar to those in allergic asthma and red-cedar induced asthma patients. A reduced level of T-lymphocytes was seen in the exposed subjects but was not observed in allergic asthma and red-cedar induced asthma patients. A variety of gasses can be emitted in a pulpmill setting including chlorine and chlorine dioxide (Kennedy *et al.*, 1991).

Courteau *et al.* (1994) evaluated 281 pulp mill construction workers, 257 of which were exposed to an average of 25 acute gassing episodes in addition to an average of 24 evacuations over a period of three to six months. The average age of the workers 44 years. Twenty-four of the 281 workers were not exposed to chlorine gas at any time during their employment. Of the 257 exposed workers, 52 had left the construction site due to health problems caused by irritant gases. Workers (including the 52 that left) were evaluated on a retrospective basis, using health records and questionnaires to collect information on individual worker exposures. Smoking histories and pre-existing conditions were recorded.

Symptoms that were associated with each worker's most significant incident of chlorine gas exposure included eye and throat irritation and cough with a frequency of 67-78% (throat and cough symptoms had a mean duration of 8-11 days and eye irritation had a mean duration of 2 days). Also prevalent were the symptoms of a flu like syndrome, headache, nose and sinus congestion, cough, fatigue and shortness of breath, with a frequency of 53-63% (a mean duration of 7-14 days for the above symptoms except the flu). Additional symptoms included difficulty sleeping (37 %), nausea (36%), excessive sweating and distaste for smoking (30%) and abdominal pain (20%). Symptoms typically lasted 1-3 weeks. Researchers categorized the workers into low and high risk groups for developing chronic lung disease as a result of the repeated chlorine gas exposure, and those workers were enrolled in a prospective study (Bherer *et al.*, 1994).

Of the 438 air samples taken in the bleach plant, 36% were <0.5 ppm, 58% were 0.5-8 ppm and 6% were >8 ppm. Experts who examined the air sample data reported that the samples were taken after workers had been evacuated and that >65% of the samples were invalid due to technical flaws and errors.

Bherer *et al.* (1994) conducted a follow up study of the Quebec pulp mill research done by Courteau and associates over a time interval of 18-24 months after the incidents of repeated exposures. Fifty-eight of the original 289 exposed workers from the moderate to high risk group were studied for developing reactive airways dysfunction syndrome (RADS). Workers at a moderate risk were defined as having shortness of breath after their most significant exposure, but not at the time of the initial study by Corteau *et al.* Moderate risk workers also had a record of other significant medical conditions and/or were 50 years of age or older. High risk workers were defined as those experiencing shortness of breath that continued one month after the exposure and/or abnormal lung sounds. Ninety percent of the follow up group completed questionnaires which revealed a 91% incidence of respiratory symptoms. Spirometry assessments and methacholine inhalation tests were conducted on 51 of the 58 workers. Twenty-three percent of the 58 workers still experienced bronchial obstruction and 41% continued to have bronchial hyper-responsiveness. Lower baseline FEV_1 was seen in those with a lower PC_{20}, and 52% of these workers showed an $FEV_1 < 80\%$ predicted.

Enarson *et al.* (1984) compared 392 pulpmill workers exposed to chlorine (unspecified duration) to a comparable group of 310 rail yard workers living in the same community, but not exposed to chlorine. In the pulpmill areas surveyed that predominantly had significant chlorine gas levels (machine room and bleach plant), workers were exposed to either an average of 0.02 ppm or 0.18 ppm Cl_2 respectively. Of the machine room workers, 23.2% experienced a cough as did 32.8% of those in the bleach plant, compared to 22.3% of the control rail yard workers. Chest tightness occurred in 31.5% of the machine room workers and 39.6% of the bleach plant workers as compared to 21.3% of the control. Only data from Caucasian subjects were reported.

Chester *et al.* (1969) evaluated 139 workers occupationally exposed to <1 ppm chlorine for an unspecified duration. Fifty-five of the 139 workers were exposed to additional accidental high concentrations of chlorine which were severe enough to require oxygen therapy. Ventilation was shown to be affected by chlorine inhalation, with a decrease in the maximal midexpiratory flow (MMF). Smokers in this group had significantly reduced FVC, FEV_1 and MMF compared to nonsmokers. Fifty-six of the 139 subjects showed abnormal posteroanterior chest films, 49 of which had parenchyma and/or hilar calcifications consistent with old granulomatus disease and 11 of which had multiple, bilateral and diffuse calcifications. Researchers suggest that the first ventilation function affected in obstructive airway disease is MMF.

A case report by Donnelly (1990) described an incident of reactive airways dysfunction syndrome in a thirty year old man following his exposure to chlorine gas. His symptomatic, clinical and physiological evidence of airway obstruction persisted after 6 years, suggesting that RADS patients can experience acquired persistent asthmatic symptoms.

Rea *et al.* (1989) exposed fifty individuals previously categorized as “chemically sensitive” to <0.33 ppm chlorine gas under double blind challenge conditions. The patients were between the ages of 21-61 and had a variety of vascular, asthmatic and arthritic conditions. Primary signs, symptoms and pulse rate were recorded before the exposure, immediately after and every 15 minutes for four hours after the exposure. Each patient was observed for specific symptoms connected with his/her sensitivity as well as other general signs. Of the 100 patients originally

screened for this study, 50 of them were excused as they were too sensitive to withstand the 15 minutes of exposure that was required, thus leaving 50 moderately sensitive patients to be involved in the research. Four of the 50 patients experienced two of the three following responses to exposure to <0.33 ppm chlorine: an increase in pulse rate beyond three standard deviations over the baseline; appearance of primary signs and symptom response (unspecified) of ≥20% over the baseline; and no response to placebo measured by primary signs, symptoms or statistically significant increase in pulse rate. The LOAEL for this study was unquantified, but was below 0.33 ppm.

In a study by Gautrin *et al.* (1994) in which acute reversibility of reactive airways dysfunction syndrome (RADS) was compared to that of occupational asthma (OA) with a latency period, chlorine inhalation appeared to cause RADS in 12 of the 15 subjects evaluated. The subjects showed FEV_1 of <80% of the predicted value and had a history of acute symptoms which occurred minutes to hours after accidental chlorine exposure. Asthma symptoms persisted after the initial symptoms disappeared.

V. Effects of Exposure to Animals

Wolf *et al.* (1995) exposed male and female B6C3F1 mice and F344 rats to chlorine gas concentrations of 0 ppm, 0.4 ppm, 1.0 ppm and 2.5 ppm. The exposures were carried out for 104 weeks at 6 hr/day 3 days/week for female rats and 6 hr/day 5 days/week for mice and male rats. Based on previous studies, the authors determined that female rats could not tolerate 5 days/week exposure to chlorine. Each treatment group contained 320 male and 320 female mice. The rats were studied in groups of 70, yielding 280 per gender per species. For the first 13 weeks of observation, body weights and clinical observations were noted weekly, and for the remainder of the study, they were recorded once every two weeks. After 52 weeks, 10 rats were euthanized and autopsied. Organ weights were recorded, and hematologic and clinical chemistry parameters were determined. These same measurements were performed on all of the surviving mice and rats at the conclusion of the 104 weeks. Male mice exposed to 1.0 and 2.5 ppm Cl_2 showed decreased weight gain compared to controls while only female mice exposed to 2.5 ppm Cl_2 showed decreased weight gain. Male rats showed decreased weight gain at all levels of exposure while female rats showed the same result at only 1.0 and 2.5 ppm Cl_2 exposures. Various nonneoplastic nasal lesions were seen in all the airway epithelial types in the nose and at all levels of exposures for both species. These lesions were evaluated against background lesions found in the control animals. A statistically significant incidence of fenestration was seen in all three exposure concentrations of Cl_2. Statistically significant responses were seen in the traditional and respiratory epithelial regions of all exposed rats and mice. Statistically significant damage to olfactory epithelium occurred in all exposed rats and female mice and also in the 1.0 and 2.5 ppm exposed groups of male mice.

Klonne *et al.* (1987) exposed 32 male and female rhesus monkeys to chlorine gas for one year to measured concentrations of 0, 0.1, 0.5, and 2.3 ppm Cl_2. These monkeys were exposed to chlorine for 6 hours/day, 5 days/week. The monkeys were evaluated periodically on the basis of body weight, electrocardiograms, neurologic examinations, pulmonary function, hematologic

parameters, serum chemistry, urinalysis, and blood gas and pH levels. Results were compared to the same test measurements recorded prior to the study. No significant difference was seen in body weight at any point in the experiment. Ocular irritation (tearing, rubbing of the eyes, reddened eyes) was observed after 6 weeks of exposure in the 2.3 ppm group. No exposure-related differences were seen in neurologic examinations, electrocardiograms, clinical chemistry, urinalysis, hematology or blood gas levels. Also, no exposure-related changes were observed in the parameters of ventilation distribution. Pulmonary function evaluations yielded a statistically significant trend for increasing pulmonary diffusing capacity and distribution of ventilation values for males and females in the 2.3 ppm exposure group. Both males and females of the 2.3 ppm group exhibited statistically significant increased incidence of respiratory epithelial hyperplasia. A mild form of the lesions was also seen in the 0.5 ppm group, 0.1 ppm group (females only) and one male in the control group. Two parasitic infections occurred, affecting the respiratory tract and resulting in 11 monkeys housing parasites and/or ova. Additionally, 16 monkeys displayed histologic changes characteristic of the presence of the parasites. However, the parasitic induced lesions were not associated with lesions in the respiratory epithelium.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Wolf *et al.*, 1995
Study population	Female F344 rats (70 per group)
Exposure method	Discontinuous whole-body inhalation exposure (0, 0.4, 1.0 or 2.5 ppm)
Critical effects	Respiratory epithelial lesions (see following table)
LOAEL	0.4 ppm
NOAEL	Not established
Exposure continuity	6 hours/day, 3 days/week, MWF
Average experimental exposure	0.043 ppm for LOAEL group
Human equivalent concentration	0.0069 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.16 based on BW = 229 g, MV = 0.17 L/min, SA(ET) = 15 cm^2)
Exposure duration	2 years
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.02 ppb (0.06 $\mu g/m^3$)

The Wolf *et al.* (1995) study was chosen as the key reference for the chlorine chronic REL for several reasons. First, the duration of the experiment was for a full lifetime of two years. Second, the sample sizes were large (280 per sex per species). Finally, appropriate sensitive endpoints of respiratory epithelial damage were examined. The mice and male rats were exposed

to chlorine for 6 hours/day, 5 days/week, but the female rats were only exposed for 3 days/week as the authors observed the females to be more sensitive than the males. Table 1 shows the histological findings of the female rats. Statistically significant results ($p < 0.05$) were seen for all the tissues at 0.4 ppm chlorine exposure and above.

Table 1. Female Rat Epithelial Lesions following Chronic Chlorine Exposure (Wolf *et al.*, 1995)

Tissues	0 ppm	0.4 ppm	1.0 ppm	2.5 ppm
Goblet cell hyperplasia	3/70 (4%)	50/70 (71%)	63/70 (90%)	64/70 (91%)
Respiratory epithelium eosinophilic accumulation	49/70 (70%)	60/70 (85%)	59/70 (84%)	65/70 (93%)
Glandular epithelium eosinophilic accumulation	16/70 (23%)	28/70 (40%)	52/70 (75%)	53/70 (76%)
Olfactory epithelium eosinophilic accumulation	36/70 (52%)	64/70 (91%)	69/70 (99%)	69/70 (99%)

The Wolf *et al.* (1995) study was chosen over the Klonne *et al.* (1987) monkey study for the following reasons: the monkeys were exposed for only one year of their total 35 year lifetime, and the sample sizes were considerably smaller (4 monkeys per sex per group) than the mouse and rat groups (280 per sex per species). Although the exposure durations differed between the two studies, the histological results were similar, differing only slightly in the region of occurrence. The monkeys displayed both tracheal and nasal lesions. Both the rodents and the monkeys showed upper respiratory epithelial lesions, thus suggesting that the rodents may be an appropriate model for humans.

For comparison, a benchmark dose analysis was performed using a log-normal probit analysis (Tox-Risk, version 3.5; ICF-Kaiser Inc., Ruston, LA) of the female rat data. Using the data for glandular epithelial eosinophilic accumulation to derive the BMC_{05} resulted in a 3-fold lower value than the LOAEL observed above, or $BMC_{05} = 0.14$ ppm. A BMC_{05} or BMC_{10} has been considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

Adequate benchmark dose estimates could not be obtained for the other nasal lesions due to high background rates and shallow dose-response relationships.

The strengths of the inhalation REL include the availability of chronic multiple-dose inhalation exposure data from a well-conducted study with histopathological analysis. Major areas of uncertainty are the lack of adequate human exposure data, the lack of observation of a NOAEL, and limited reproductive toxicity data.

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CHRONIC TOXICITY SUMMARY

CHLORINE DIOXIDE

(Anthium dioxcide; Alcide; chlorine oxide; chlorine peroxide; chloryl radical; doxcide 50)

CAS Registry Number: 10049-04-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.2 μg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Vascular congestion and peribronchiolar edema; Hemorrhagic alveoli and congested capillaries in the lung in rats
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Molecular formula	ClO_2
Molecular weight	67.64 g/mol
Specific gravity	1.642 @ 0°C (liquid)
Boiling point	11° C
Vapor pressure	Unknown
Solubility	Soluble in water, alkaline and sulfuric acid solutions
Description	Yellow to red liquid or gas
Conversion factor	1 ppm = 2.76 mg/m^3

III. Major Uses or Sources

Chlorine dioxide is used as a bleaching agent for cellulose, textiles, flour, leather, oils, and beeswax. It is also used in the purification of water and as a bactericide and antiseptic (HSDB, 1994).

IV. Effects of Human Exposures

Case reports of human occupational exposure to chlorine dioxide have shown that 19 ppm was fatal to one worker and 5 ppm was definitely irritating (Elkins, 1959). Seven out of 12 workers exposed regularly to chlorine dioxide at levels generally below 0.1 ppm (0.28 mg/m^3) reported symptoms of ocular and respiratory irritation leading to slight bronchitis (Gloemme and

Lundgren, 1957). Concurrent exposure to chlorine and chlorine dioxide in pulp mill workers resulted in an increase in the reporting of subjective symptoms of irritation (Ferris *et al.*, 1967). In this study, the chlorine dioxide concentrations ranged from trace levels to 0.25 ppm (0.69 mg/m^3). No differences were found between these workers and controls for pulmonary function tests.

V. Effects of Animal Exposures

Eight rats (sex unspecified) were exposed for 5 hours/day, 5 days/week, for 2 months to 0 or 1 ppm (2.8 mg/m^3) chlorine dioxide (Paulet and Debrousses, 1972). The number of control animals was not specified. Microscopic evaluation of the lungs revealed vascular congestion and peribronchiolar edema in all animals exposed to chlorine dioxide. The LOAEL for respiratory effects was therefore 1 ppm (2.8 mg/m^3). An earlier study by these researchers (Paulet and Debrousses, 1970) examined the effects of exposure to 2.5, 5, or 10 ppm chlorine dioxide for several hours/day for 30 days in rats and rabbits (n = 4-10 animals per group). Body weights, blood cell counts, and histopathological examination of the liver, lungs, and other tissues were measured in each group. At 10 ppm, nasal discharge, localized bronchopneumonia, and desquamated alveolar epithelium were observed. White and red blood cell counts were also increased with this exposure. Rats and rabbits exposed to 2.5 ppm for 7 hours/day for 30 days or for 4 hours/day for 45 days, respectively, showed significant respiratory effects, including hemorrhagic alveoli and inflammatory infiltration of the alveolar spaces.

Rats exposed to 5, 10, or 15 ppm (13.8, 27.6, or 41.4 mg/m^3) chlorine dioxide for 15 minutes, 2 or 4 times/day, for 1 month showed an increase in congested lungs, nasal discharge, and catarrhous lesions of the alveoli beginning at 10 ppm (Paulet and Debrousses, 1974). No significant changes in these parameters were seen at 5 ppm.

Dalhamn (1957) found that acute exposure to 260 ppm chlorine dioxide for 2 hours resulted in 1 death out of 4 rats. Five out of 5 rats died during exposures of 4 hours/day for 14 days. All exposed animals exhibited signs of respiratory distress and ocular discharge. No effects were seen in 5 rats exposed to 0.1 ppm for 5 hours/day, 7 days/week, for 10 weeks.

VI. Derivation of U.S. EPA RfC

Study	Paulet and Debrousses (1970, 1972); U.S. EPA, 1995
Study population	Wistar rats (8 per exposure concentration)
Exposure method	Discontinuous whole-body inhalation (0 or 1 ppm)
Critical effects	Vascular congestion; peribronchial edema; lung alveolar damage
LOAEL	1 ppm
NOAEL	Not observed
Exposure continuity	5 hours/day, 5 days/week
Exposure duration	2 months
Average experimental exposure	0.15 ppm for LOAEL group
Human equivalent concentration	0.23 ppm for LOAEL group (gas with thoracic respiratory effects, RGDR = 1.57 based on MV = 0.17 m^3, SA(Th) = 3,460 cm^2)
LOAEL uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Subchronic factor	10
Modifying factor	3 (database deficiencies)
Cumulative uncertainty factor	3,000
Inhalation reference exposure level	0.00008 ppm (0.08 ppb, 0.0002 mg/m^3, 0.2 μg/m^3)

There were uncertainties in all areas of concern. Thus the best available study still was limited by lack of multiple exposure concentrations, the relatively short duration of exposures, the small number of animals examines, and the lack of adequate human health effects information. lack of dose-response information, and the the lack of comprehensive multi-organ effects data.

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CHRONIC TOXICITY SUMMARY

2-CHLOROACETOPHENONE

(Phenacyl chloride, CN, mace)

CAS Registry Number: 532-27-4

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.03 µg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Squamous hyperplasia of the nasal respiratory epithelium in rats.
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (HSDB, 1994)

Molecular formula	C_8H_7ClO
Molecular weight	154.60
Description	Colorless to gray crystalline solid
Boiling point	244-245° C
Melting point	56.5° C
Vapor pressure	0.0054 mm Hg @ 20° C
Solubility	Soluble in alcohol, benzene, ether; practically insoluble in water
Conversion factor	6.32 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

2-Chloroacetophenone (CAP) is a potent lacrimator found in mace (HSDB, 1994). It is also used as an alcohol denaturant and as a pharmaceutical intermediate. CAP has been synthesized by chlorination of acetophenone with selenium oxychloride (NTP, 1990).

IV. Effects of Human Exposure

Numerous reports on the acute inhalation toxicity of 2-chloroacetophenone (CAP) are available due to its extensive use as a tear gas agent. Symptoms of acute toxicity reported by human volunteers exposed briefly to a range of concentrations (40-350 mg/m^3) include tingling of the nose, rhinorrhea, burning of the throat and eyes, lacrimation, blurred vision (Punte *et al.*, 1962).

Burning in the chest, dyspnea, slight gagging, and nausea were also reported. Slight transient increases in airway resistance were also measured in the exposed subjects.

V. Effects of Animal Exposure

Rats and mice were exposed to CAP for 6 hours per day, 5 days per week for 103 weeks (NTP, 1990). Dose-related lesions of the respiratory epithelium were observed in rats exposed to 1 or 2 mg/m^3 CAP. A possible viral infection, as indicated by positive tests for antibodies for several rat viruses, may have exacerbated the response in rats to the respiratory irritant effects of CAP. The only compound-related clinical sign observed during the exposure was eye irritation.

The RfC review indicates that no information on the toxicokinetics of inhaled CAP or its reproductive or developmental toxicity was located (U.S. EPA, 1994).

VI. Derivation of U.S. EPA RfC

Study	NTP, 1990; U.S. EPA (1994)
Study population	Rats; mice (60 per species per group per sex)
Exposure method	Discontinuous whole-body inhalation exposures (0, 1, or 2 mg/m^3)
Critical effects	Squamous hyperplasia of the nasal epithelium of rats
LOAEL	1 mg/m^3
NOAEL	Not observed
Exposure continuity	6 hours/day, 5 days/week
Average experimental exposure	0.18 mg/m^3 for LOAEL group
Human equivalent concentration	0.03 mg/m^3 for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.18 based on MV = 0.24 m^3/day, SA(ET) = 11.6 cm^2)
Exposure duration	103 weeks
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Modifying factor	3 (lack of neurotoxicity and reproductive toxicity data)
Cumulative uncertainty factor	1,000
Inhalation reference exposure level	0.03 $\mu g/m^3$ (0.005 ppb)

Strengths of the CAP REL include the availability of multiple-species, multiple-dose chronic inhalation studies and the observation of a mild effect LOAEL.

The major uncertainties are the lack of human data and the lack of a NOAEL observation. IRIS reported medium confidence in the study, low confidence in the adequacy of the data base and low confidence in the RfC. Another weakness of the REL arises from the the authors suggested that the irritant effects of CAP may have been exacerbated by viral infection; sentinel and control animals were positive for antibodies to several rat viruses at 6, 12, 18, and 24 months. Inflammation, ulcers, and squamous hyperplasia observed in the forestomach of exposed female rats may have been due to ingestion of CAP during fur grooming.

The strengths of the inhalation REL include the availability of chronic inhalation exposure data from a well-conducted study in multiple species with histopathological analysis. Major areas of uncertainty are the lack of human data and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

CHLOROBENZENE

(Synonym monochlorobenzene; benzene chloride; benzene monochloride; chlorbenzene; chlorbenzol; phenyl chloride)

CAS Registry Number: 108-90-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**1000 μg/m^3**
Critical effect(s)	Increased liver weights, hepatocellular hypertrophy, renal degeneration and inflammation, and testicular degeneration in rats
Hazard index target(s)	Alimentary system; kidney; reproductive system

II. Physical and Chemical Properties Summary

Molecular formula	C_6H_5Cl
Molecular weight	112.56 g/mol (HSDB, 1994)
Description	Colorless, neutral liquid (HSDB, 1994)
Vapor pressure	11.8 mm Hg at 25^{o}C (HSDB, 1994)
Solubility	Practically insoluble in water (0.049 g/100 ml); soluble in alcohol, benzene, chloroform, diethyl ether (HSDB, 1994)
Conversion factor	1 ppm = 4.60 mg/m^3 at 25 ^{o}C

III. Major Uses and Sources

As one of the most widely utilized chlorinated benzenes, mono-chlorobenzene has been a major chemical for 50 years. Historically important in the manufacture of chlorinated pesticides, especially DDT, and in the production of phenol and aniline, monochlorobenzene's principal current use is as a chemical intermediate in the production of chemicals such as nitrochlorobenzenes and diphenyl oxide. These chemicals are subsequently used in the production of herbicides, dyestuffs and rubber chemicals. Additionally, monochlorobenzene is used as a solvent in degreasing processes (e.g., in metal cleaning operations), paints, adhesives, waxes and polishes (HSDB, 1995; NIOSH, 1993).

IV. Effects of Human Exposure

Even though monochlorobenzene has been industrially utilized for several years, few epidemiologic and/or occupational studies have addressed the potential health status of workers chronically exposed to monochlorobenzene (NIOSH, 1993). A Russian occupational study (Rozenbaum *et al.*, 1947, as reported by the U.S. EPA, 1988) describes multiple central nervous system effects after intermittent exposure to monochlorobenzene in a mixed chemical environmental over 2 years, including headache, numbness, dizziness, cyanosis, hyperesthesia, and muscle spasms. No specific exposure levels or histopatholoigic data were reported.

Two small studies utilizing volunteers exposed to single doses of monochlorobenzene have reported central nervous system effects (Ogata *et al.*, 1991; Tarkhova, 1965). A exposure chamber study of five volunteers exposed up to 60 ppm monochlorobenzene (276 mg/m^3) for a single 7 hour exposure described acute subjective symptoms such as drowsiness, headache, eye irritation and sore throat (Ogata *et al.*, 1991). One other human volunteer study described altered electrical activity of the cerebral cortex in four individuals exposed to 43.4 ppm monochlorobenzene vapors for 2.5 minutes (Tarkhova, 1965).

V. Effects of Animal Exposure

No chronic inhalation studies have evaluated the toxicity of monochlorobenzene. Only a single, oral chronic carcinogenicity study (NTP, 1985) has evaluated the long-term adverse affects of monochlorobenzene administration. However, a few subchronic inhalation studies have demonstrated adverse effects on the liver, the kidney, and to a lesser extent, blood parameters following monochlorobenzene exposure over a period of weeks or months (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987).

One subchronic study evaluated Sprague-Dawley male rats and rabbits exposed to 0, 75, or 200 ppm of monochlorobenzene for 7 hr/day, 5 days/week, for up to 24 weeks (Dilley, 1977). In rats, monochlorobenzene-related toxicity included increased absolute and relative (to brain- or body-weight) organ weights (especially the liver) after 11 and 24 weeks of exposure (LOAEL 75 ppm). Male rabbits also demonstrated increases in liver weight after 24 weeks of exposure (LOAEL 75 ppm). Some hematological changes in red blood cell parameters were reported in rats including differences in platelet and reticulocyte counts between control and exposed animals; however, some changes observed at 11 weeks were variable and comparable to controls at 24 weeks (red blood cell count, hemoglobins, hematocrit, and white blood cell count). Pathological changes were observed in rats, with occasional focal lesions in the adrenal cortex, tubular lesions in the kidneys, and congestion in the liver and kidneys.

Two other subchronic inhalation studies reported adverse organ effects following monochlorobenzene exposure in rats and rabbits (John *et al.*, 1984; Nair *et al.*, 1987). In the first study, John *et al.* (1984) reported increased liver weights in rats and rabbits following short-term (10 or 13 day, 6 hours/day) monochlorobenzene exposure, LOAEL 590 ppm in rats and 210 ppm in rabbits. Nair *et al.* (1987) exposed male and female Sprague-Dawley rats to 0, 50, 150 or 450

ppm monochlorobenzene vapors daily for 6 hours over 10-11 weeks prior to mating, and up to day 20 of gestation for 2 generations and found dose-related changes in the livers, kidneys, and testes in both generations of males (F0 and F1). Hepatotoxicity occurred as hepatocellular hypertrophy and increased liver weights (mean and absolute) at concentrations greater than 50 ppm (LOAEL 150 ppm). At this concentration, renal changes included tubular dilation, interstitial nephritis, and foci of regenerative epithelium. Testicular degeneration of the germinal epithelium occurred in both generations of exposed males, but no chlorobenzene-induced adverse effects on reproductive performance or fertility were seen.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Nair *et al.* (1987)
Study population	Sprague-Dawley rats (30/sex/group)
Exposure method	Discontinuous inhalation exposures (0,50,150,450 ppm)
Critical Effects	Increases in absolute and relative liver weights (F_0 and F_1 both sexes), hepatocellular hypertrophy (F_0 and F_1 males), renal degeneration and inflammation (F_0 and F_1 both sexes), testicular degeneration (F_0 and F_1 males).
LOAEL	150 ppm
NOAEL	50 ppm
Exposure continuity	6 hours/day, 7 days/week
Exposure duration	11 weeks
Average experimental exposure	13 ppm for NOAEL group
Human equivalent concentration	26 ppm (gas with systemic effects, based on RGDR = 2.0 for lambda (a) : lambda (h)) (Gargas *et al*., 1989)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	3
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.3 ppm (300 ppb; 1.0 mg/m^3, 1000 $\mu g/m^3$)

Of the three inhalation studies available (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987), the Nair *et al.* (1987) two generational developmental study was selected for identifying a NOAEL and LOAEL as it best presented the histopathology of the adverse effects, and demonstrated a dose response relationship for these effects (statistically significant increases in mean liver weights, incidence of renal changes, and testicular degeneration).

Another subchronic inhalation study (Dilley, 1977) also observed increases in organ weights, including the liver, in rats after 11 and 24 weeks exposure to 75 and 250 ppm

monochlorobenzene (LOAEL 75 ppm), and, in rabbits at 24 weeks. Similar adverse liver and kidney effects found in subchronic oral bioassays (Kluwe *et al.*, 1985; NTP, 1985) include increases in liver weight and hepatocellular degeneration in rats (LOAEL 125 mg/kg/day) and mice (250 mg/kg/day); and, renal necrosis and degeneration in rats (LOAEL 500 mg/kg/day) and mice (LOAEL 250 mg/kg/day) after 13 weeks oral exposure to chlorobenzene.

Uncertainty factors are appropriate due to the lack of chronic studies, both animal bioassay and human, and, the limited number of subchronic inhalation studies; thereby requiring determination of the chronic REL from this shorter term, single species study. The magnitude of interspecies variation remains unknown, as few species have been tested and human data for comparison remains lacking. However, metabolic studies have demonstrated species variation in the urinary elimination of chlorobenzene metabolites (Ogata and Shimada 1983; Ogata *et al.*, 1991; Yoshida *et al.*, 1986). Humans metabolize and excrete chlorobenzene predominately as free and conjugated forms of 4-chlorocatechol and chlorophenols, while the main rodent urinary metabolite p-chlorophenylmercapturic acid, is found in minor amounts (<0.5%). No information exists identifying possible susceptible human subpopulations to monochlorobenzene exposure.

The strengths of the inhalation REL include the observation of a NOAEL, the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and the demonstration of a dose-response relationship. Major areas of uncertainty are the lack of adequate human exposure data and limited reproductive toxicity data.

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CHRONIC TOXICITY SUMMARY

CHLORODIFLUOROMETHANE

(freon-22; fluorocarbon-22; hydrochlorofluorocarbon; FC 22; CFC 22; monochlorodifluoromethane)

CAS Registry Number: 75-45-6

I. Chronic Toxicity Summary

Inhalation reference exposure level	**50,000 μg/m^3** (U.S. EPA-RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC
Critical effect(s)	Decreased weight gain in male Alderley Park Wistar-derived rats; increased weight of kidney, adrenal and pituitary gland in female Alderley Park Wistar-derived rats; hyperactivity in male Alderley Park Swiss-derived mice. Increased incidence of anophthalmia in CD rat fetuses.
Hazard index target(s)	Teratogenicity; kidney; endocrine system

II. Chemical Property Summary (HSDB, 1995)

Molecular formula:	$CHClF_2$
Molecular weight:	86.47
Description:	Colorless denser-than-air gas; nearly odorless, like carbon tetrachloride.
Vapor pressure:	10 mmHg at -103.7°C.
Solubility:	Moderate water solubility, 2770 mg/l at 25°C. Soluble in ether, acetone and chloroform.
Conversion factor:	3.54 μg/m^3 per ppb at 25°C

III. Major Uses and Sources

Major uses of chlorodifluoromethane (Freon 22) are as a refrigerant, low-temperature solvent, coolant in air-conditioning systems, aerosol propellant and to a small degree as a blowing agent for polyurethane resins (HSDB, 1995). Production of Freon 22 rose 5% in 1994 and accounts for over 50% of current fluorocarbon production in the U.S.(C&EN, 1995). Due to scheduled decreases in production of fully halogenated chlorofluorocarbons regulated by the Montreal

Protocol (Freon 11, 12, 113, 114), production of Freon 22, unregulated so far, is expected to grow substantially to fill the voids created by the protocol. The ozone-depleting and global-warming potentials of the partially halogenated hydrocarbons, such as Freon 22, are considerably lower and their atmospheric residence times are shorter (Anders, 1991). Freon 22 may be released to the environment during its production, storage, transport and use. Exposure to low levels of Freon 22 occurs by inhalation of ambient air. Occupational exposure may occur via inhalation and dermal contact with the vapor or liquefied gas.

IV. Effects of Human Exposures

The pharmacokinetics of CFCs, including Freon 22, are characterized by rapid pulmonary absorption and distribution. There is no indication of any accumulation (WHO, 1991,). Following exposure of human volunteers to 0.32 or 1.81 g/m^3 for 4 hr, three phases of elimination were present during the post-exposure period (Woollen *et al.*, 1992). The half-lives were 3 min, 12 min, and 2.7 hr, and probably corresponded to elimination from alveolar air and/or lung tissues, elimination from well perfused tissues and elimination from poorly perfused tissues, respectively. Metabolic transformation of Freon 22 was not detected. Therefore, toxic effects of metabolites are very unlikely. Regardless of the route of entry, CFCs appear to be eliminated almost entirely through the respiratory route.

No chronic adverse effects have been recorded in humans. Occupational studies of 539 workers exposed to CFCs, including Freon 22, during construction and repairing of refrigeration equipment for up to 10 years found no increase in death rates among the workers (Szmidt *et al.*, 1981). In another study, 89 workers were examined during their work with refrigerant equipment involving Freon 22 in 32% of the cases. No effects were seen regarding cardiac arrythmias and reaction time measurements (Edling and Olsen, 1988; Edling *et al.*, 1990).

V. Effects of Animal Exposures

In the rat, a direct correlation was found between inhaled and the blood concentrations of Freon 22 (Carney, 1977). When exposure was stopped, clearance from the bloodstream was very rapid, with a half-life of about 3 minutes. Similar results were seen in the rabbit (Sakata *et al.*, 1981). Blood concentration was directly proportional to the inhaled concentration of Freon 22 and plateaued within 5 minutes of initiation of exposure. A 50% decrease in blood concentration occurred within 1 minute of cessation of exposure. Studies with radiolabelled Freon 22 indicated that metabolism in the rat was minimal (Salmon *et al.*, 1979). The amount of $^{14}CO_2$ released was no more than 0.1% of the inhaled Freon 22. The amounts of ^{14}C label in the urine was similarly very small with insignificant amounts excreted in feces.

Subchronic inhalation studies with Freon 22 produced little or no effects. A 4 week study in which rats, guinea pigs, dogs and cats were exposed to 175 g/m^3 (50,000 ppm) for 3.5 hr/day observed no clinical, biochemical or pathological effects (Weigand, 1971). A 13 week study exposing rats to 35 and 17.5 g/m^3 (10,000 and 5000 ppm, respectively) and beagle dogs to 17.5

g/m^3 for 6 hr/day saw no changes in behavior, body weight, hematology, clinical biochemistry and organ weights (Leuschner *et al.*, 1983). In an 8 week study, Lee and Suzuki (1981) exposed two groups of 16 male rats to 175 g/m^3 Freon 22 for 5 hr/day. No adverse effects were observed on 6 of the rats submitted for hematological, biochemical and histopathological analysis at the end of the study. The remaining rats were immediately incorporated into a one-generation male fertility study with unexposed female rats. Other than a slight decrease in prostate weight, no other organs were affected. Plasma glucose and triglyceride levels were slightly reduced and plasma cholesterol slightly raised, but no hematological parameter was affected. Overall, there was no effect on male fertility or dominant lethality.

An early study by Karpov (1963) exposed rats, mice and rabbits to 50 g/m^3 Freon 22, as well as rats and mice to 7 g/m^3, for 6 hr/day, 6 days/week for 10 months. A number of effects were seen at 50 g/m^3 including, depressed body weight gain in mice after 2-4 months, depressed oxygen consumption in rats, CNS function changes in rats and mice, decreased hemoglobin concentration in rabbits and histopathological (dystrophic) changes in the liver, lungs and nervous tissue. No effect was seen at the lower exposure level (7 g/m^3).

The most comprehensive long-term Freon 22 inhalation study was performed by Tinston *et al.* (1981a, 1981b). Alderley Park Swiss-derived mice and Alderley Park Wistar-derived rats (80 rats or mice/sex/group, including two control groups) were exposed to 0, 3.5, 35 and 175 g/m^3 (0, 1000, 10,000 and 50,000 ppm) for 5 hr/day, 5 days/week until 80% mortality occurred (female mice, 83 weeks; male mice, 94 weeks; female rats, 118 weeks; male rats, 131 weeks). No chronic adverse effects were noted in mice with the exception of hyperactivity observed in males exposed to 175 g/m^3. In rats at the highest dose, males exhibited decreased body weight gain up to week 80, but significant differences (up to 12%) in weight occurred mainly in the first 24 weeks of the study. Female rats exhibited increased kidney, adrenal and pituitary gland weights, all in excess of 10% of control values. Females also had slightly (<10% of controls) increased liver weights. All other chronic exposure indices were within normal parameters.

In comprehensive reproductive studies, female CD rats were exposed to 0, 100, 1000 or 50,000 ppm Freon 22 for 6 hr/day on days 6-15 of gestation (Palmer *et al.*, 1978a). Nineteen batches of time-mated females were used, each batch being made up of 34 control rats and 22 rats in each exposure group. This enabled the examination of more than 6000 control fetuses and more than 4000 fetuses from each exposure group. Other than decreased body weight gain at the highest dose, no maternal effects were observed. In fetuses, there was a significant increase in the incidence of anophthalmia in the 50,000 ppm group. Microphthalmia was also seen at this dose level but was not significant compared to controls. Other reproductive indices were normal. A similar reproduction study performed in female New Zealand White rabbits did not find a significant increase in eye malformations or any other teratogenic effects in fetuses (Palmer *et al.*, 1978b). There were no signs of treatment-related effects in Freon 22-exposed females and pregnancy was normal.

The overall assessment from reproduction and lifetime studies (using modern protocols) in a variety of species indicates that repeated exposure to Freon 22 at a high concentration of

50,000 ppm (175 g/m^3) has only small teratogenic effects and noncancer chronic effects (Litchfield and Longstaff, 1984).

VI. Derivation of U.S. EPA Reference Concentration (RfC)

Study	Tinston *et al.*, 1981a,b; Palmer *et al.*, 1978a,b
Study population	80 rats or mice/group/sex with 2 control groups/sex; 800 total animals of each species (Tinston *et al.*, 1981a,b). Over 6000 control group rat fetuses and over 4000 rat fetuses in each exposure group (Palmer *et al.*, 1978a).
Exposure method	Discontinuous whole body inhalation exposure (1000, 10,000 or 50,000 ppm for Tinston studies; 100, 1000 or 50,000 for Palmer study).
Critical effects	General body system (in mice, hyperactivity in males. In rats, decreased body weight gain in males; increased kidney, adrenal and pituitary weight in females) and teratogenicity (increased incidence of anophthalmia in CD rat fetuses.)
LOAEL	50,000 ppm
NOAEL	10,000 ppm
Exposure continuity	5 hr/day, 5 days/week (Tinston studies); 6 hr/day during gestational days 6-15 (Palmer study).
Exposure duration	Female mice, 83 weeks; male mice, 94 weeks; female rats, 118 weeks; male rats, 131 weeks
Average experimental exposure	1488 ppm
Human equivalent concentration	1488 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies factor	10
Modifying factor	3 (database deficiencies, including lack of a two-generation reproductive study)
Cumulative uncertainty factor	100
Inhalation reference exposure level	20 ppm (20,000 ppb, 50 mg/m^3, 50,000 μg/m^3).

Long-term exposure of rats and mice to Freon 22 resulted in mostly mild adverse effects at 50,000 ppm (Tinston *et al.*, 1981a,b). Exposure of female rats to Freon 22 during gestation, also at 50,000 ppm, resulted in a weak teratogenic effect (Palmer *et al.*, 1978a). Based on these studies, the LOAEL for Freon 22 was established at 50,000 ppm. The NOAEL is 10,000 ppm and is based on the long-term exposure study by Tinston *et al.* (1981a,b). The average experimental exposure for continuous exposure, as opposed to intermittent exposure used in the study (5 hr/day, 5 days/week), was calculated to be 1488 ppm. Applying uncertainty factors of

10 each to account for interspecies differences and to account for any increased susceptibility of sensitive human populations, an inhalation reference exposure level of 20 ppm (50 mg/m^3) was estimated.

The long-term studies by Tinston *et al.* (1981a, 1981b) are supported by subchronic studies which found relatively mild toxicity (at g/m^3 levels) for Freon 22 in several different species. Moreover, in the few human studies of occupational exposure to CFCs that included Freon 22, no adverse effects were observed. Experimental animals and humans presented similar pharmacokinetcs of Freon 22, rapid half-life upon cessation of exposure and little or no metabolism.

Weaknesses of the long-term study include endpoints for mouse exposure that were not in the Tinston *et al.* (1981a) report. For example, data on organ weights were missing. There was also no definition of hyperactivity or scoring criteria for determining hyperactivity seen in male mice. While a one generation study investigating male fertility has been performed, a two-generation study has yet to be done for Freon 22.

The strengths of the inhalation REL include the availability of chronic inhalation exposure data from a well-conducted study with histopathological analysis, the demonstration of a dose-response relationship, and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, and limited reproductive toxicity data.

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CHRONIC TOXICITY SUMMARY

CHLOROFORM

(trichloromethane; formyl trichloride; methenyl trichloride; methyl trichloride)

CAS Registry Number: 67-66-3

I. Chronic Toxicity Summary

Inhalation reference exposure level	**300 µg/m^3**
Critical effect(s)	Liver toxicity (degenerative, foamy vacuolization, and necrosis in rats; increased liver weights) in male rats Kidney toxicity (cloudy swelling and nephritis) in rats Developmental toxicity
Hazard index target(s)	Alimentary system; kidney; teratogenicity

II. Chemical Property Summary (HSDB, 1995)

Molecular formula	$CHCl_3$
Molecular weight	119.49 g/mol
Description	Colorless liquid
Vapor pressure	200 mm Hg 25^{o}C
Solubility	Soluble in water (8220 g/L); miscible in carbon tetrachloride, carbon disulfide alcohols, benzene, ethers and oils
Conversion factor	4.9 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Chloroform ($CHCl_3$) is used in industry and laboratory settings as a solvent for adhesives, pesticides, fats, oils and rubbers. It is also used as a chemical intermediate in the synthesis of fluorocarbon 22, dyes, pesticides, and tribromomethane. Chloroform is produced as a byproduct of water, sewage, and wood pulp chlorination (HSDB, 1995).

IV. Effects of Human Exposure

Limited information is available regarding possible adverse health effects in humans following chronic inhalation of chloroform. However, historical clinical reports from patients who underwent chloroform anesthesia indicate that acute inhalation exposure affects the central nervous system, cardiovascular system, stomach, liver, and kidneys (Schroeder, 1965; Smith *et al.*, 1973; Whitaker and Jones, 1965). Acute chloroform toxicity included impaired liver function (Smith *et al.*, 1973), toxic hepatitis (Lunt, 1953; Schroeder, 1965), cardiac arrhythmia (Payne, 1981; Schroeder, 1965; Whitaker and Jones, 1965), nausea (Schroeder, 1965; Smith *et al.*, 1973, Whitaker and Jones, 1965), central nervous system symptoms (Schroeder, 1965; Whitaker and Jones, 1965). Chronic inhalation studies are limited to a few occupational studies identifying the liver and the central nervous system as target organs (Challen *et al.*, 1958; Li *et al.*, 1993; Phoon *et al.*, 1983; Bomski *et al.*, 1967).

Challen *et al.* (1958) investigated workers manufacturing throat lozenges with exposure to chloroform vapors estimated in the range 77 to 237 ppm with episodes of >1100 ppm. Workers reported symptoms of fatigue, dull-wittedness, depression, gastrointestinal distress, and, frequent and burning micturition. No evidence of liver dysfunction was found based on thymol turbidity, serum bilirubin, and urine urobilinogen levels.

Bomski *et al.* (1967) reported 17 cases of hepatomegaly in a group of 68 chloroform exposed workers. Chloroform concentrations ranged from 2 to 205 ppm (duration 1 to 4 years). Three of the 17 workers with hepatomegaly had toxic hepatitis based on elevated serum enzymes. Additionally, 10 workers had splenomegaly. Workers exposed to chloroform had a 10-fold increased risk of contracting viral hepatitis compared to the general population. The study authors considered the chloroform induced liver toxicity as a predisposing factor for viral hepatitis, but the incidence of viral hepatitis in the workers is in itself a confounding factor.

Phoon *et al.* (1983) described two outbreaks of toxic jaundice in workers manufacturing electronics equipment in Singapore. One plant had 13 cases of jaundice, initially diagnosed as viral hepatitis, in a work area with >400 ppm chloroform. Blood samples from workers (five with jaundice, four without symptoms) contained between 0.10 and 0.29 mg chloroform/100 mL. A second factory reported 18 cases of hepatitis, all from a work area utilizing chloroform as an adhesive. Two samplings indicated air levels of 14.4 to 50.4 ppm chloroform. Due to a lack of fever and hepatitis B surface antigen in the patients, the authors attributed the jaundice to chloroform exposure rather than viral hepatitis.

More recently, Li *et al.*(1993) reported on chloroform exposed workers from a variety of production factories. Exposure levels varied widely, from 4.27 to 147.91 mg/m^3 (119 samples), with 45% of the samples below 20 mg/m^3. The authors' report that exposed workers displayed altered neurobehavioral function and liver damage (abnormal activities serum enzymes).

These cross sectional studies are limited in their ability to establish chronic NOAEL/LOAEL values due to limited exposures, concurrent exposure to other chemicals, inadequate control

groups and potential confounders. However, these studies indicate the potential for liver and central nervous system toxicity in humans exposed to chloroform via inhalation.

V. Effects of Animal Exposure

Exposure of experimental animals to chloroform for acute, subchronic or chronic durations results in toxicity to the liver and kidney, as well as respiratory and central nervous systems (USDHHS, 1993). The majority of chronic animal studies have used oral routes of chloroform administration (USDHHS, 1993), while only limited data is available on inhalation specific exposures. Both routes of exposure, however, appear to primarily affect the liver and kidney (Chu *et al.*, 1982; Heywood *et al.*, 1979; Jorgenson *et al.*, 1985; Miklashevshii *et al.*, 1966; Munson *et al.* 1982; Roe *et al.*, 1979; Torkelson *et al.*, 1976).

Torkelson and associates (1976) exposed rats (12/sex/group), rabbits (2-3/sex/group), and guinea pigs (8-12/sex/group) for 7 hours/day, 5 days/week over 6 months to 0, 25, 50 or 85 ppm chloroform vapor. Dogs were exposed to 25 ppm chloroform, for 7 hours/day, 5 days/week for 6 months. Dose and species-dependent pathological changes in the liver included mild to severe centrilobular granular degeneration, foamy vacuolization, focal necrosis, and fibrosis in both sexes of all species tested. Guinea pigs were the least sensitive and male rats the most sensitive to chloroform induced hepatotoxicity with the above adverse effects occurring at 25 ppm. Adverse kidney effects observed in all species included cloudy swelling of the renal tubular epithelium and interstitial and tubular nephritis. Pneumonitis was observed in the high (85 ppm) exposure groups of male rats, female guinea pigs, and male rabbits, and in the lower dose group of female rabbits (25 ppm). Clinical and blood parameters were also examined in rats and rabbits, but no alterations were attributable to chloroform exposure.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Torkelson *et al.*(1976)
Study population	Rats, unspecified strain (12/sex/group)
Exposure method	Discontinuous whole-body inhalation exposures (0, 25, 50, 85 ppm)
Critical effects	Pathological changes in liver (degenerative), kidneys (cloudy swelling)
LOAEL	25 ppm
NOAEL	Not observed
Exposure continuity	7 hr/day for 5 days/week, for 6 months
Average experimental exposure	5.3 ppm for LOAEL group
Human equivalent concentration	15.9 ppm for LOAEL group (gas with systemic effects, based on RGDR = 3.0 for lambda (a) : lambda (h) (Gargas, *et al.* 1989))
Exposure duration	6 months
LOAEL uncertainty factor	10

Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.05 ppm (50 ppb; 0.30 mg/m^3; 300 µg/m^3)

In the study of Torkelson and associates (1976) rats were the most sensitive species and guinea pigs the least sensitive to chloroform vapors. Though of subchronic duration, this inhalation study still exposed rats discontinuously for 25% of a lifetime (25.8 weeks/104 weeks/lifetime). Pathological changes were observed in both sexes of rat at 50 and 85 ppm (244 or 415 mg/m^3) and in male rats at 25 ppm (122 mg/m^3) chloroform. These hepatic changes included mild to severe centrilobular granular degeneration, foamy vacuolization, focal necrosis, and fibrosis. Adverse effects in the kidney including cloudy swelling and nephritis were seen in all species tested at 25 ppm (122 mg/m^3) chloroform.

The human occupational studies have reported jaundice with or without alterations in liver enzymes at similar ambient concentrations, 2 to 204 ppm chloroform (10 to 995 mg/m^3) after at least 1 year (Bomski *et al.*, 1967) and 14 to 400 ppm chloroform (68 to 1952 mg/m^3) after 6 months or less (Phoon *et al.*, 1983).

Chloroform is metabolized by the cytochrome P-450 dependent mixed function oxidase system primarily in the liver, the respiratory epithelium, and the kidney. In the rat liver and kidneys, chloroform is metabolized to phosgene (Pohl *et al.*, 1984). The hepatotoxicity and nephrotoxicity of chloroform is thought to be due largely to phosgene (Bailie *et al.*, 1984). Individuals with concurrent exposure to certain chemical inducers of liver cytochrome P450 activity, including barbiturates, may be at potentially greater risk of chloroform toxicity (Cornish *et al.* 1973). Others with possible higher sensitivity to chloroform include persons with underlying liver, kidney or neurological conditions.

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CHRONIC TOXICITY SUMMARY

CHLOROPICRIN

(trichloronitromethane; nitrochloroform; nitrochloromethane)

CAS Registry Number: 76-06-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**4 µg/m^3**
Critical effect(s)	Increased mortality, increased lung and liver weight, nasal rhinitis
Hazard index target(s)	Respiratory system, alimentary system

II. Chemical Property Summary (from HSDB (1995) except as noted)

Molecular formula	CCl_3NO_2
Molecular weight	164.4 g/mol
Description	Colorless to faint yellow liquid
Boiling point	112°C
Vapor pressure	5.7 mm Hg @ 0°C; 24 mm Hg @ 25°C (Fries and West, 1921)
Solubility	1.6 g/l water @ 25°C; 2.272 g/l water @ 0°C 1.9 g/l water @ 20°C; miscible with benzene, ethanol, carbon disulfide, ether, carbon tetrachloride, acetone, methanol, acetic acid
Conversion factor	6.72 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Chloropicrin is primarily used as a fumigant against insects and fungi in grain elevators and storage bins (HSDB, 1995). It has also been used in the fumigation of non-deciduous fruit trees and produce. Chloropicrin is used as an indicator chemical in other fumigants such as methyl bromide because of its potent irritational properties. Chloropicrin kills weed and grass seeds when applied to soil. Chloropicrin was used in World War I as a chemical warfare agent because of its potent activity as a lachrymator. Chloropicrin has a minor use in the chemical synthesis of methyl violet. Chloropicrin can also form in drinking water as a result of chlorination processes (Duguet *et al.*, 1985; Merlet *et al.*, 1985).

IV. Effects of Human Exposure

No studies are available which describe toxic effects to humans from chronic exposure to chloropicrin. Human exposures to concentrations less than 1 ppm for very short periods of time are extremely irritating (ACGIH, 1992; Fries and West, 1921). The threshold of odor detection in humans is approximately 1 ppm (ACGIH, 1992).

V. Effects of Animal Exposure

Burleigh-Flayer and Benson (1995) conducted an inhalation bioassay with CD rats (60 per sex per dose) exposed discontinuously to 0, 0.1, 0.5 or 1.0 ppm chloropicrin 6 hours/day for 5 days/week over 107 weeks. Increased mortality was noted in males at 0.5 and 1 ppm. Increased lung and liver weight and nasal rhinitis were reported at the 1 ppm level.

Male Swiss-Webster mice (group numbers ranging from 16-24) were exposed by inhalation to a single level of different sensory irritants including chloropicrin for 6 hours/day for 5 days, with unexposed control groups of 8-10 mice (Buckley *et al.*, 1984). The exposure level for chloropicrin was 7.9 ppm, which approximated the level sufficient to cause a 50% decrease in respiratory rate in mice (RD50) (Kane *et al.*, 1979). Half the exposed mice and half the control animals were sacrificed immediately after the exposures and the other half 72 hours after the last exposure. All were examined for respiratory tract lesions. Body weights of chloropicrin exposed animals were reduced 10-25% below controls, but increased to normal levels during the recovery period. Nasal exudate and distention of the abdomen were observed. "Moderate" lesions characterized by exfoliation, erosion, ulceration, or necrosis were observed in the respiratory and olfactory epithelium and minimal inflammation and squamous metaplasia were observed in the respiratory epithelium alone. Moderate to severe damage to the lower respiratory tract was described as "fibrosing peribronchitis and peribronchiolitis". Exfoliation, hyperplasia, and squamous metaplasia were also noted.

A study of the toxicity of chloropicrin by oral exposure in Sprague-Dawley rats was conducted (Condie *et al.*, 1994). Ten and ninety-day studies were conducted dosing animals daily with chloropicrin in vehicle (corn oil) at a volume of 1 ml/kg. Groups of 10 rats/sex/group were dosed with 0, 10, 20, 40, and 80 mg/kg for the 10-day study and with 0, 2, 8, and 32 mg/kg for the 90-day study. Parameters examined included mortality, body weight, food and water consumption, hematology, serum clinical chemistry, gross pathology and histology of organs. Only the high-dose group and the control group animals from the 90-day study were examined histopathologically. In the 90-day study, 6 males and 2 females in the 32 mg/kg dose group and 1 male and 3 females in the 8 mg/kg dose group died before the scheduled sacrifice time. The authors note signs of pulmonary complications (inflammation and congestion) in the dead animals. Previously, the animals had shown signs of respiratory distress, including wheezing and dyspnea. The deaths were considered to be exposure related and most likely due to aspiration of chloropicrin. Among the survivors, mean body weight, hemoglobin levels, and hematocrit were significantly reduced in males in the 32 mg/kg dose group. Absolute thymus weights were reduced in female rats at 32 mg/kg, and female rats in the 8 mg/kg dose group showed decreased

white blood cell count. Most animals in the 32 mg/kg dose group (>60%) showed histopathological changes in the forestomach including chronic inflammation, acantholysis, and hyperkeratosis.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Burleigh-Flayer and Benson (1995)
Study population	CD rats (60 per sex per dose)
Exposure method	Discontinuous inhalation (0, 0.1, 0.5 or 1.0 ppm)
Critical effects	Increased mortality, increased lung and liver weight, nasal rhinitis
LOAEL	0.5 ppm
NOAEL	0.1 ppm
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	107 weeks
Average experimental exposure	0.018 ppm for NOAEL group
Human equivalent concentration	0.018 ppm for NOAEL group
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	30
Inhalation reference exposure level	0.0006 ppm (0.6 ppb; 4 $\mu g/m^3$; 0.004 mg/m^3)

Significant strengths in the REL include the duration of exposure (lifetime), the multiple dose study design with adequate sample sizes, and the demonstration of a NOAEL.

Major areas of uncertainty are the lack of adequate human exposure data and limited reproductive toxicity data.

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CHRONIC TOXICITY SUMMARY

HEXAVALENT CHROMIUM (SOLUBLE COMPOUNDS)

Molecular Formula	***Molecular Weight***	***Synonyms***	***CAS #***
CrO_3	99.99 g/mol	Chromic trioxide, chromium oxide, chromium trioxide, chromium (VI) oxide	1333-82-0
$K_2CrH_2O_4$	194.20 g/mol	Potassium chromate, dipotassium chromate, potassium (VI) chromate, dipotassium monochromate, chromate of potash	7789-00-6
Li_2CrO_4	129.87 g/mol	Lithium chromate, dilithium salt, chromium lithium oxide, chromic acid, lithium chromate (VI)	14307-35-8
$Na_2CrH_2O_4$	161.97 g/mol	Sodium chromate, chromic acid disodium salt, chromium disodium oxide, sodium chromate (VI), chromate of soda	7775-11-3
$K_2Cr_2H_2O_7$	294.20 g/mol	Potassium dichromate, dichromic acid dipotassium salt, bichromate of potash	7778-50-9
$Na_2Cr_2H_2O_7$	261.96 g/mol	Sodium dichromate, bichromate of sodium, chromic acid disodium salt, chromium sodium oxide	10588-01-9

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.0008 µg/m^3 Cr(VI)**
Critical effect(s)	Respiratory effects (nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes) in human occupational study
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

Description	Dark red or brown solid
Molecular formula	See above
Molecular weight	See above
Specific gravity	2.70 @ 25°C
Boiling point	Not found
Melting point	197 °C
Vapor pressure	Not found
Solubility	Soluble in water, ethyl alcohol, ethyl ether, sulfuric and nitric acid
Conversion factor	Not applicable

III. Major Uses or Sources

Hexavalent chromium is more toxic than Cr (III), the form most commonly found naturally (ATSDR, 1987). While more information is available on the toxicity of soluble Cr (VI) compounds, information on insoluble Cr (VI) compounds has been included where applicable.

Chromates are used in paints, cooling towers, locomotives, and automobiles to inhibit metal corrosion due to recirculating water. Sources of chromium emission include sewage sludge, municipal incineration, and chemical manufacture. Chromic acid, used to electroplate metal parts, is the most common hexavalent chromium compound produced (NAS, 1988).

IV. Effects of Human Exposure

Workers exposed to 2 $\mu g/m^3$ Cr(VI) as chromic acid for a mean of 2.5 years exhibited an increased incidence of nasal atrophy, nasal mucosal ulcerations, and nasal septal perforations as compared to controls (Lindberg and Hedenstierna, 1983). The same study reported statistically significant decreases in FEV_1, FVC, and FEF_{25-75} measurements taken on a Thursday afternoon as compared to those taken on a Monday morning in nonsmoking workers exposed to 2 $\mu g/m^3$ Cr(VI) or more. Similar changes were observed in the smokers although only FVC measured on a Thursday was statistically significant. Because no significant differences were observed between pulmonary function measurements for exposed and unexposed workers taken on a Monday morning (prior to a work-week of exposure), the authors infer that the observed pulmonary function changes are transient.

Gastritis and duodenal ulcers, in addition to ulceration and perforation of the nasal septum, were observed in chrome platers exposed to a mean breathing zone concentration of 4 $\mu g/m^3$ chromic acid for an average of 7.5 years (Lucas and Kramkowski, 1975).

Male workers in the chromate and dichromate production industry whose occupational exposures were 0.05-1.0 mg Cr(VI)/m^3 as chromium trioxide for a mean of 7 years were reported to have

elevated levels of low molecular weight proteins (retinol binding protein and tubular antigens) in the urine (Franchini and Mutti, 1988). The authors suggest that the presence of such proteins in the urine is an early indicator of kidney damage.

V. Effects of Animal Exposure

Rats exposed to 200 µg/m^3 Cr(VI) as sodium dichromate by inhalation for 22 hours per day, for 42 days exhibited decreased alveolar macrophage phagocytic activity; the lung clearance of inert iron oxide was significantly reduced in exposed rats compared to controls (Glaser *et al.*, 1985). Increased alveolar macrophage activity and a significantly elevated antibody response to injected sheep red blood cells were observed in rats exposed to 25 or 50 µg/m^3 Cr(VI) for 22 hours per day for 28 days.

A later experiment exposed male rats to 0, 50, 100, 200, or 400 µg Cr/m^3 22 hours per day, 7 days per week for 90 days (Glaser *et al.*, 1989). Bronchoalveolar lavage fluid contained elevated levels of albumin, LDH, and total protein in all exposed groups. Statistically significant elevations in these parameters were observed mainly in the 200 and 400 µg/m^3 exposure groups. At necropsy, a statistically significant increase in lung weight was observed in rats exposed to 100, 200, and 400 µg/m^3 as compared to controls. An analysis of this data (Malsch *et al.*, 1994) determined the benchmark dose (95% confidence interval with dose associated with a 10% elevation in the parameter) for each of these endpoints. The analysis also examined changes in lung and spleen weight reported in Glaser *et al.*, 1985. The most sensitive endpoint was LDH in BALF.

Nasal septal perforation, hyperplastic and metaplastic changes in the larynx, trachea and bronchus and emphysema were observed in mice exposed two days per week for 12 months to CrO_3 mist in concentrations of either 3.63 mg/m^3 for 30 minutes per day or 1.81 mg/m^3 for 120 minutes per day (Adachi, 1987; Adachi *et al.*, 1986).

VI. Derivation of Chronic Reference Exposure Level (REL)

Derivation of Chronic Inhalation Reference Exposure Level

Study	Lindberg and Hedenstierna, 1983
Study population	Human workers
Exposure method	Occupational exposure
Critical effects	Nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes
LOAEL	Average occupational exposure of 1 µg/m^3 Cr(VI) as chromic acid, with a range between non-detectable concentrations (< 0.2 µg/m^3) and 2 µg/m^3

NOAEL	Not observed
Exposure continuity	8 hours per day, 5 days per week
Exposure duration	Mean of 2.5 years (range = 0.2 - 23.6 years)
Average exposure	0.24 µg/m^3 Cr(VI)
Human equivalent concentration	0.24 µg/m^3 Cr(VI)
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.0008 µg/m^3 Cr(VI)

The human exposure study of Lindberg and Hedenstierna (1983) was selected as the best available human study. Also, the available animal studies had shortcomings that limited their usefulness. A 10-fold uncertainty factor was applied for the lack of a NOAEL observation. The mean exposure duration (3% of lifetime) was less than the 8 to 12% of lifetime that has been used to differentiate chronic from subchronic studies. However, since exposure ranged up to 26 years, a factor of 3 was considered adequate.

The major strength of the REL is the use of adequate human data. The major uncertainties are the lack of controlled and quantified exposure data, the lack of an observation of a NOAEL, the lack of dose-response information, and the lack of comprehensive multi-organ effects data.

Derivation of Chronic Oral Reference Exposure Level

Study	Mackenzie *et al.*, 1958
Study population	8 male and 8 female Sprague-Dawley rats
Exposure method	Drinking water
Critical effects	No adverse effects seen
LOAEL	None
NOAEL	2.4 mg/kg-day (converted from 25 mg/L of chromium as K_2CrO_4)
Exposure continuity	Continuous
Exposure duration	1 year
Average experimental exposure	0.11 ppm chromium VI
LOAEL uncertainty factor	1
Subchronic uncertainty factor	5
Interspecies uncertainty factor	10
Intraspecies factor	10
Cumulative uncertainty factor	500
Oral reference exposure level	0.005 mg/kg bw-day

*Conversion Factors: Drinking water consumption = 0.097 L/kg/day (reported)

The oral REL is the U.S. EPA's oral Reference Dose (RfD) (IRIS, 1996). Groups of eight male and eight female Sprague-Dawley rats were supplied with drinking water containing 0-11 ppm (0-11 mg/L) hexavalent chromium (as K_2CrO_4) for 1 year. The control group (10/sex) received distilled water. A second experiment involved three groups of 12 males and 9 female rats. One group was given 25 ppm (25 mg/L) chromium (as K_2CrO_4); a second received 25 ppm chromium in the form of chromic chloride; and the controls again received distilled water. No significant adverse effects were seen in appearance, weight gain, or food consumption, and there were no pathologic changes in the blood or other tissues in any treatment group. The rats receiving 25 ppm of chromium (as K_2CrO_4) showed an approximate 20% reduction in water consumption. This dose corresponds to 2.4 mg chromium(VI)/kg/day based on actual body weight and water consumption data.

For rats treated with 0-11 ppm (in the diet), blood was examined monthly, and tissues (livers, kidneys and femurs) were examined at 6 months and 1 year. Spleens were also examined at 1 year. The 25 ppm groups (and corresponding controls) were examined similarly, except that no animals were killed at 6 months. An abrupt rise in tissue chromium concentrations was noted in rats treated with greater than 5 ppm. The authors stated that "apparently, tissues can accumulate considerable quantities of chromium before pathological changes result." In the 25 ppm treatment groups, tissue concentrations of chromium were approximately 9 times higher for those treated with hexavalent chromium than for the trivalent group.

Similar no-effect levels have been observed in dogs and humans. Anwar *et al.* (1961) observed no significant effects in female dogs (2/dose group) given up to 11.2 ppm chromium(VI) (as K_2CrO_4) in drinking water for 4 years. The calculated doses were 0.012-0.30 mg/kg of chromium(VI). In humans, no adverse health effects were detected (by physical examination) in a family of four persons who drank for 3 years from a private well containing chromium(VI) at approximately 1 mg/L (0.03 mg/kg/day for a 70-kg human).

This RfD is limited to soluble salts of metallic chromium(VI). Examples of soluble salts include potassium dichromate ($K_2Cr_2O_7$), sodium dichromate ($Na_2Cr_2O_7$), potassium chromate (K_2CrO_4) and sodium chromate (Na_2CrO_4). Trivalent chromium is an essential nutrient. There is some evidence to indicate that hexavalent chromium is reduced in part to trivalent chromium in vivo (Petrilli and DeFlora, 1977, 1978; Gruber and Jennette, 1978). The literature available on possible fetal damage caused by chromium compounds is limited. No studies were located on teratogenic effects resulting from ingestion of chromium.

The uncertainty factor of 500 represents two 10-fold decreases in dose to account for both the expected interhuman and interspecies variability in the toxicity of the chemical in lieu of specific data, and an additional factor of 5 to compensate for the less-than-lifetime exposure duration of the principal study.

U.S. EPA stated its confidence in the RfD as: Study - Low; Data Base - Low; and RfD -- Low. Confidence in the chosen study is low because of the small number of animals tested, the small number of parameters measured and the lack of toxic effect at the highest dose tested.

Confidence in the data base is low because the supporting studies are of equally low quality, and teratogenic and reproductive endpoints are not well studied. Low confidence in the RfD follows.

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CHRONIC TOXICITY SUMMARY

COBALT

(Cobalt (II) chloride hexahydrate, cobalt blue, cobalt chloride, cobaltous chloride)

CAS No. 7440-480-43

COBALT SULFATE

(Cobalt sulfate heptahydrate, cobaltous sulfate)

CAS Registry Number: 10026-24-1

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.005 µg/m^3**
Critical effect(s)	Respiratory effects in rats and mice
Hazard index target(s)	Respiratory system

II. Physical and Chemical Properties (HSDB, 1995)

Description	Gray, hard, malleable metallic solid
Molecular formula	Co
Molecular weight	58.93 g/mol
Specific gravity	8.92 @ 20ºC
Boiling point	2870ºC
Melting point	1493ºC
Vapor pressure	0 mm Hg at 20ºC
Conversion factor	Not applicable

III. Major Uses or Sources

Cobalt is a natural element which can result in exposures through air, food, and water. Cobalt is used in the manufacture of steel alloys, abrasives, and pigments. It has been used in electroplating processes; as a drier for printing inks, paints and varnishes; and as a catalyst (ATSDR, 1992). In 1990, 7,512 metric tons of cobalt were consumed in the United States (HSDB, 1995). The average concentration of cobalt in ambient air in the United States is approximately 0.0004 µg/m^3. However, ambient concentrations as high as 0.6 µg/m^3 have been reported (ATSDR, 1992).

IV. Effects of Exposures to Humans

A cohort of 319 cobalt-exposed Japanese metal factory workers was studied retrospectively. Airborne cobalt concentrations ranged from 0.003 to 1.3 mg/m^3. Mean duration of exposure was 10.3 years and the follow-up period was 4 years. Eighteen hard metal exposed workers (5.6%) had occupational asthma. Nine of the 18 had positive bronchial provocation tests with cobalt and 2 of these 9 had positive cobalt patch tests. The remaining 9 declined to be tested (Kusaka *et al.*, 1986a).

Fifteen healthy previously unexposed men were exposed over a single 6 hour interval to hard metal dust containing cobalt (mean of 38 µg/m^3) (Kusaka *et al.*, 1986b). Forced vital capacity was significantly decreased ($p<0.05$), although there was no significant correlation with cobalt concentrations.

Among 42 workers exposed to an average 85 µg cobalt/m^3 over 7 hours from hard metal dust, no significant decreases in ventilatory function were detected (Kusaka *et al.*, 1986b). Among the same 42 workers exposed earlier to a mean concentration of 126 µg cobalt/m^3 the forced expiratory volume at 1 second (FEV_1)had been earlier significantly ($p< 0.05$) depressed compared with matched controls.

Another group of 35 workers was exposed to unknown concentrations of cobalt for unknown durations (Bencko *et al.*, 1986). The geometric mean of hair cobalt concentration in exposed workers (96.8 µg/g) was significantly higher than in the control group (0.4 µg/g). Cobalt-exposed workers had a significant elevation in IgA ($p< 0.05$) and a significant decrease in IgE ($0.001< p< 0.05$). Exposed workers had significant elevations in the concentration of α-antitrypsin, α_2-macroglobulin, ceruloplasmin, and lysozyme.

Progressive lung disease was observed among 12 workers exposed to cobalt powder (Friberg *et al.*, 1986). Cobalt exposures exceeded 100 µg/m^3 and occurred over periods of 1 month to 28 years. Lung fibrosis, cough, wheezing and shortness of breath were also observed. Eight of the workers died, and were found to have developed pulmonary interstitial infiltration and fibrosis.

Of 247 factory workers exposed to 0.8 to 12 mg cobalt/m^3, 8 developed pulmonary changes (Kaplun, 1957). Nausea, abdominal pains, loss of appetite, cough, and loss of odor perception were also reported. Some also had decreased hemoglobin levels and red blood cell counts, liver and spleen enlargement, and dermatitis. Of 117 workers studied further, 30% had chronic bronchitis, 29% had fibrotic changes, and 44% had decreased blood pressure.

Cobalt has been used as a foam stabilizer in beer (NIOSH, 1981). Between 1964 and 1966, 1 to 1.5 ppm cobaltous chloride was added to 20-25% of beer produced in the United States. Around the same time, several epidemics of an uncommon cardiomyopathy occurred among heavy beer drinkers. Symptoms observed included gastrointestinal disorders, labored breathing, abdominal pain, cyanosis, lowered blood pressure, heart enlargement, pericardial effusion, rapid heart rate, and electrocardiographic abnormalities. Cobalt salts have been implicated in certain forms of cardiac disease.

Trace amounts of cobalt are needed in the human diet. However, the daily human requirement for cobalt is unknown because there is no data on cobalt limited diets sufficient to cause adverse effects (HSDB, 1995). Effects would presumably be analogous to that of vitamin B12 deficiency. Average daily dietary intakes have been estimated to be between 0.005 and 2 mg. The average human body burden of cobalt in a 70 kg person is estimated to be approximately 3 mg.

V. Effects of Exposures to Animals

Cobalt sulfate heptahydrate aerosol was administered F344/N rats and B6C3F1 mice for 6 hours per day, 5 days per week over 16 days or 13 weeks (Bucher *et al.*, 1990). In the short-term studies, all rats and mice exposed at 200 mg cobalt sulfate/m^3 died while partial survival was seen in the 50 mg/m^3 groups. Nasal olfactory epithelium degeneration and necrotizing nasal inflammation were noted in all rats and mice exposed to 50 mg/m^3 or more. Peribronchial and septal fibrosis in the lung were observed at 50 mg/m^3. Necrotizing inflammation was noted in the larynx and trachea of rats and mice at 5 mg/m^3.

In the 13-week studies, animal were exposed to 0, 0.3, 1, 3, 10, or 30 mg/m^3 (Bucher *et al.*, 1990). Lung weights were increased over those of controls in rats exposed to 1 mg/m^3 or more and in mice exposed to 10 mg/m^3 or more. Polycythemia was observed in rats only. Sperm motility was decreased in mice exposed at the lowest dose tested for that effect (3 mg/m^3). Increased abnormal sperm and reduced testis and epididymal weights were found in mice at 30 mg/m^3. Respiratory tract lesions in rats and mice included degeneration of the olfactory epithelium, respiratory epithelium squamous metaplasia, and inflammation in the nose; inflammation, necrosis, squamous metaplasia, and inflammatory polyps in the larynx; squamous metaplasia in the trachea; and histiocytic infiltrates, bronchiolar regeneration, peribronchiolar and septal fibrosis, and alveolar epithelial hyperplasia in the lung. The larynx was the most sensitive site, with squamous metaplasia noted in both rats and mice at 0.3 mg/m^3, the lowest exposure tested. Even the lowest concentration tested was associated with a high incidence of adverse effects (see table below).

Incidence of squamous metaplasia of the larynx following 13 weeks inhalation

	0 mg/m^3	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
Rats	0/10	9/10	10/10	10/10	10/10	10/10
Mouse	0/10	7/10	10/10	5/9	9/10	10/10

Rats were exposed continuously to cobalt metal aerosols (50 or 500 µg/m^3) for 45 or 90 days. Foci of epithelial hyperplasia were found in thyroid gland follicles of rats exposed to 500 µg/m^3. Changes in thyroid function were observed in animals exposed to 50 µg/m^3 (Popov *et al.*, 1977) .

Miniature swine inhaled cobalt metal powder aerosol (0.1 or 1.0 mg cobalt/m^3) for 6 hr/day, 5 days/wk over 3 months (Friberg *et al.*, 1986). A dose-dependent decease in ventilatory lung compliance was noted among exposed animals. Thickening and collagenization of alveolar septa was reported.

Rats were administered subcutaneously cobalt chloride (1 mg/kg, 5 mg/kg, 20 mg/kg and 50 mg/kg bw) daily for 8 days (Hatori *et al.*, 1993). Manganese-superoxide dismutase (Mn-SOD) activity was significantly reduced in the cobalt exposed groups, while glutathione peroxidase activity was increase. Myocardial cobalt concentrations and Mn-SOD activity were inversely related.

Male mice were continuously exposed to cobalt (400 ppm) via drinking water over 13-weeks (Anderson *et al.*, 1992). A consistent pattern was observed of seminiferous tubule degeneration with initial Sertoli cell vacuolation and formation of abnormal spermatid nuclei, the appearance of multinucleated cells and sloughing of cells, and tubule shrinkage with accumulation of calcified necrotic material.

Rabbits inhaled 500 µg/m^3 Co as $CoCl_2$ with or without 500 µg/m^3 Ni as $NiCl_2$ for 6 hours per day, 5 days per week over 4 months (Johansson *et al.*, 1991). Nickel exposure potentiated cobalt-induced formation of type II cells nodules.

VI. Derivation of Chronic Reference Exposure Level

Study	Bucher *et al.* (1990)
Study population	F344/N rats and B6C3F1 mice (10/group/sex)
Exposure method	Discontinuous whole-body inhalation exposures to 0, 0.3, 1, 3 10, or 30 mg/m^3 cobalt sulfate heptahydrate aerosols
Critical effects	Squamous metaplasia of the larynx in both species
LOAEL	0.3 mg/m^3 cobalt sulfate heptahydrate for both species
NOAEL	Not observed
Exposure continuity	6 hours/day for 5 days/week
Average experimental exposure	0.054 mg/m^3 for LOAEL group
Human equivalent concentration	0.0045 mg/m^3 for LOAEL group (particulate with extrathoracic respiratory effects, female rat RDDR = 0.084, based on MMAD = 0.96 µm, sigma g = 1.04, BW = 229 g, MV = 0.17 L/min, SA(ET) = 15 cm^2)
Exposure duration	13 weeks
LOAEL uncertainty factor	10
Subchronic uncertainty factor	3
Interspecies uncertainty factor (UF)	3
Intraspecies uncertainty factor (UF)	10

Cumulative uncertainty factor	1,000
Inhalation reference exposure level	0.005 μg/m^3

Available studies of human exposure to cobalt were inadequate for estimation of a chronic inhalation reference exposure level. While several epidemiological studies have shown adverse respiratory effects among workers exposed to 7 to 900 μg cobalt/m^3 over 2 to 17 years (ATSDR, 1992), limitations with each of these studies outweighed those of more complete controlled animal studies.

Strengths of the REL include: (1) known, controlled exposures were administered over a significant fraction of lifetime, (2) two species studied developed similar adverse effects, and (3) it was a well-designed study that involved comprehensive clinical and pathological examinations.

Uncertainties in the chronic REL include: (1) the high incidence of adverse effects at the lowest concentration tested in the key study and lack of a clear NOAEL in this or other studies, (2) the study involved less than lifetime exposures, (3) lack of human exposure studies adequate for risk assessment, and (4) the lack of reproductive and developmental toxicity studies.

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CHRONIC TOXICITY SUMMARY

COPPER AND COPPER COMPOUNDS

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Cu	copper	63.55	7440-50-8
$CuCl_2$	copper chloride	134.45	7447-39-4
CuO	cupric oxide; copper oxide; copper (I) oxide	79.54	1317-38-0
$CuSO_4$	copper sulfate; blue vitrol; copper (II) sulfate; cupric sulfate; blue copper; blue stone	159.61	7758-98-7

I. Chronic Toxicity Summary

Inhalation reference exposure level	**0.02 µg Cu/m^3**
Critical effect(s)	'Metal fume fever'; cold-like symptoms in humans
Hazard index target(s)	Respiratory system

II. Chemical Property Summary (from HSDB, 1995, except as noted)

Molecular formula	see above
Molecular weight	see above
Description	Cu: reddish lustrous metal $CuCl_2$: yellow-brown powder CuO: brownish/black crystalline powder $CuSO_4$: blue crystals
Vapor pressure	1 mm Hg @ 1628°C
Solubility	Cu: insol. in H_2O; sol. in HNO_3, hot H_2SO_4; sl. sol. in HCl, NH_4OH $CuCl_2$: 706 g/l H_2O; sl. sol. in alcohol (Beliles, 1981) CuO: insol. H_2O; sol. in NH_4Cl, KCN (Beliles, 1981) $CuSO_4$: 14.3 g/100 ml H_2O @ 0°C; 75.4 g/100 ml @ 100°C; 1.04 g/100 ml CH_3OH @ 18°C
Conversion factor	Not applicable

III. Major Uses and Sources

Copper is a widely used structural metal, particularly in applications where high electrical and thermal conductivity are required, and is the major component of bronze and brass (ACGIH, 1992). Copper fume may be generated in copper and brass foundries, smelters, and in the welding of copper containing metals. Copper compounds are also found in fungicides and other agricultural products, ceramics, and pyrotechnics. Copper sulfate ($CuSO_4$) is the most common salt of copper and is used as a fungicide, a component of electroplating solutions, and as a chemical intermediate for other copper salts and dyes and in the tanning of leather. Copper oxide (CuO) is another common copper compound used in insecticides, fungicides, catalysts, fuel additives, cement, and wood preservatives.

IV. Effects of Exposures to Humans

Workers employed in sieving copper dust and copper electroplating were examined over a period of four years, with 100, 97, 75, and 97 workers examined in the four consecutive years beginning in 1970 (Suciu *et al.*, 1981). Maximum concentrations to which workers in sieving operations were exposed declined from a high of 464 mg/m^3 in 1971 to 111 mg/m^3 two years later and ultimately to 7-22 mg/m^3 through health protective measures. Normal serum copper levels, based upon measurement of 20 control workers, were found to fall between 80 and 120 μg/100 ml serum. Among exposed workers, 37-46% per year had serum copper levels over 120 μg/100 ml serum. Neuro-physiological findings included changes in EEG, memory deficiencies, paresthesia and pain, cardiovascular changes, digestive disorders, respiratory problems, and endocrine disorders. In the study, however, symptom incidence was not compared with a control group.

Exposure to copper dust was reported in workers polishing copper plates (Gleason, 1968). Three men reported symptoms of warmth or chills and head stuffiness (the classic signs of 'metal fume fever') "some weeks" after the beginning of a copper plate polishing operation. Air samples showed that concentrations in the operation area ranged from 0.030 to 0.120 mg Cu/m^3, depending on location. Aluminum was also detected as a "major" or "minor" component of the dust, depending on location. Microscopic analysis of the dust showed it was of "extreme fineness". Copper dust levels in the air were reduced to 0.008 mg/m^3 when a local exhaust ventilation system was installed. As a result, symptoms among the workers subsided.

Similar symptomatology, including fever, dyspnea, chills, headache, and nausea, was also reported among 26 workers involved in the cutting of brass pipes (Armstrong *et al.*, 1983). Elevated urinary copper levels were reported in the workers and symptoms appeared an average of 4 hours after exposure.

The nasal mucosa of 16 metal workers, 10 of whom were exposed to complex copper salt dust, and 9 construction workers were examined (Askergren and Mellgren, 1975). The dust to which the workers were exposed was predominantly comprised of 26% copper hydroxide nitrate ($Cu(NO_3)_2 \cdot 3Cu(OH)_2$), 25% copper hydroxide sulfate ($CuSO_4 \cdot 3Cu(OH)_2$), 22% copper silicate ($CuSiO_3$), and 17% CuO. Exposure levels were not quantitated. Potential duration of exposure

among the 10 sheet metal workers ranged from 1 to 60 months. Workers exposed for longer periods showed atrophic changes to the nasal mucosa, as indicated by increased vascularity with more prominent superficial blood vessels. This effect was also observed with some frequency among the other non-exposed workers. The construction workers also showed other changes in nasal mucosa (livid mucosa and mucoid secretion).

V. Effects of Exposures to Animals

Male and female CD_1 mice (47 or 48/sex/group) were exposed for 3 hours/day, 5 days/week to 0.12 mg Cu/m^3 for one week or 0.13 mg Cu/m^3 for 2 weeks in the form of $CuSO_4$ and examined for changes in host defense (Drummond *et al.*, 1986). Significantly increased mortality and decreased mean survival time were reported in mice exposed to copper for two weeks followed by challenge with an aerosol of *Streptococcus zooepidemicus*. Decreased bactericidal activity of alveolar macrophages (as % *Klebsiella pneumoniae* killed) was observed in female mice exposed for one week and both male and female mice exposed for two weeks. Two week exposure produced a significant decrease in normal tracheal epithelium in female mice and extensive areas of alveolar thickening.

Eight male rabbits were exposed to 0.6 ± 0.3 mg Cu/m^3 in the form of $CuCl_2$ for 6 hr/day, 5 days/week for 4-6 weeks, with an equal number of control rabbits exposed to filtered air only (Johansson *et al.*, 1983; Johansson *et al.*, 1984; Lundborg and Camner, 1984). The rabbits were sacrificed within 3 days of the last exposure (2 per day, 3 days/week). Examination of the lungs revealed no changes in gross appearance and no statistical difference was found between exposed and control animals with respect to histological lesions (Johansson *et al.*, 1984). Type II cells of the lung were found to be slightly increased in the exposed animals, although only the volume density of the type II cells was found to be increased significantly. Total lung phospholipid was also unchanged. Extracted alveolar macrophages from copper exposed animals had slightly increased lamellated inclusions, although no increase in cell number, protrusions, oxidative metabolic activity, particle uptake, or bactericidal activity was observed (Johansson *et al.*, 1983). Lysozyme activity in lung lavage fluid and in alveolar macrophages and their conditioned medium was unchanged between treated and control animals (Lundborg and Camner, 1984).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Gleason, 1968
Study population	Three workers
Exposure method	Occupational inhalation exposure
Critical effects	'Metal fume fever'; cold-like symptoms
LOAEL	0.030-0.120 mg/m^3
NOAEL	0.008 mg/m^3
Exposure continuity	8 hrs/day × 5 days/week
Exposure duration	Unspecified ("some weeks")
Average experimental exposure	0.002 mg/m^3 for NOAEL group
Human equivalent concentration	0.002 mg/m^3 for NOAEL group
Subchronic uncertainty factor	10
LOAEL uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.02 $\mu g/m^3$

Data indicate that the inhalation of copper produces adverse effects in occupationally exposed humans. The most useful data come from the study of Gleason *et al.* (1968) showing the appearance of symptoms similar to 'metal fume fever' among workers exposed to copper dust levels in the range of 0.03 to 0.12 mg Cu/m^3. Symptoms ceased when dust levels were reduced to 0.008 mg Cu/m^3. Thus, this level can be taken as a NOAEL in the development of the chronic REL. Adjusting the dose for work-week exposure results in an average experimental exposure of 0.002 mg Cu/m^3. An uncertainty factor of 10 was applied to adjust for the less than chronic exposure and a factor of 10 was applied to adjust for potentially sensitive human subpopulations. The resulting chronic REL is 0.02 μg Cu/m^3. Other uncertainties associated with the study include: (1) the presence of other compounds in the work environment, (2) the small study size, (3) limited breadth and reporting of toxic endpoints, and (4) limited reporting of experimental detail.

Evidence from animal studies also suggests that inhalation of copper has adverse effects on the respiratory system and increases susceptibility to bacterial infection (Johansson *et al.*, 1983; Johansson *et al.*, 1984; Drummond *et al.*, 1986). In a relatively large study (47 or 48 animals/group) of mice exposed to $CuSO_4$, decreased host resistance and histological changes to respiratory epithelium were observed following two-week exposure (Drummond *et al.*, 1986). The LOAEL for the study was observed at 0.13 mg Cu/m^3 (only a single dose level was examined for two different time periods). This exposure level is considerably above the chronic REL derived from the human data. The use of this study for the derivation of the chronic REL would result in a value below that derived from the human data primarily because of uncertainty associated with using animal data, the relatively short exposure duration, and the absence of a NOAEL. However, the cumulative uncertainty associated with the human study is more acceptable than that associated with the animal study. Therefore the data from the Gleason *et al.* (1968) study have been used for the derivation of the chronic REL.

The strengths of the inhalation REL include the use of human exposure data and the observation of a NOAEL. Major areas of uncertainty are the lack of reproductive toxicity data, the lack of chronic inhalation exposure studies, the uncertainty in estimating exposure, the potential variability in exposure concentration, and the limited nature of the study.

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CHRONIC TOXICITY SUMMARY

CRESOL MIXTURES

Compounds	*Synonyms*	*CAS Reg. No.*
cresols	cresylic acid; tricresol; hydroxytoluene; methylphenol	1319-77-3
o-cresol	1-hydroxy-2-methylbenzene; 2-hydroxytoluene; 2-methylphenol	95-48-7
m-cresol	1-hydroxy-3-methylbenzene; 3-hydroxytoluene; 3-methylphenol	108-39-4
p-cresol	1-hydroxy-4-methylbenzene; 4-hydroxytoluene; 4-methylphenol	106-44-5

I. Chronic Toxicity Summary

Inhalation reference exposure level	**4 µg/m^3**
Critical effect(s)	Alterations in bone marrow cellularity in rats
Hazard index target(s)	Circulatory system

II. Chemical Property Summary (HSDB, 1995, unless otherwise noted)

Molecular formula	C_7H_8O
Molecular weight	108.13 g/mol
Description	Colorless in pure form; yellowish, brownish-yellow, or pinkish liquid
Vapor pressure	0.299 mm Hg @ 25°C (o-cresol) 0.138 mm Hg @ 25°C (m-cresol) 0.11 mm Hg @ 25°C (p-cresol) (Chao *et al.*, 1983; Daubert and Danner, 1985) 1 mm Hg @ 38-53°C
Solubility	Soluble in 50 parts water; miscible with alcohol, benzene, ether, glycerol, petroleum ether; soluble in vegetable oils, glycol
Conversion factor	4.42 µg/m^3 per ppb at 25ºC

III. Major Uses and Sources

Cresol compounds (mixtures of the ortho-, meta- and para-isomers) can be obtained from coal tar and petroleum or synthesized by sulfonation or oxidation of toluene (HSDB, 1995). Crude cresol (commercial grade) contains approximately 20% o-cresol, 40% m-cresol, and 30% p-cresol.

Phenol and xylenols are present in small amounts as contaminants. Cresylic acid compounds are called cresol when the boiling point is below 204°C.

Cresols have a wide variety of uses including the manufacture of synthetic resins, tricresyl phosphate, salicylaldehyde, coumarin, and herbicides. Cresols also serve as components of degreasing compounds in textile scouring and paintbrush cleaners as well as fumigants in photographic developers and explosives. Cresols also function as antiseptics, disinfectants, and parasiticides in veterinary medicine. An approximate breakdown of cresol and cresylic acid use is 20% phenolic resins, 20% wire enamel solvents, 10% agricultural chemicals, 5% phosphate esters, 5% disinfectants and cleaning compounds, 5% ore flotation and 25% miscellaneous and exports.

Any combustion process which results in the generation of phenolic compounds (such as automobile exhaust or coal, wood or trash smoke) may be a potential source of exposure to cresols, although under normal conditions, low vapor pressure limits the inhalation hazard presented by cresols (HSDB, 1995).

IV. Effects of Exposures to Humans

Brief exposure to 6 mg cresol/m^3 resulted in irritation of the throat and nose, nasal constriction, and dryness in 8 of 10 subjects (Uzhdavini *et al.*, 1972).

Chemical burns may result from exposure to cresols. The lungs of humans exposed to cresols have shown signs of emphysema, edema, bronchopneumonia, and small hemorrhages (Clayton and Clayton, 1982). Skin contact has resulted in the development of white patches and blistering, eventually turning brown or black (Lefaux, 1968). Other reported effects have included turbidity, inflammation, and fatty degeneration of the liver, nephritis, and hemorrhage of the epicardium and endocardium. An infant fatally exposed to ~20 ml of a 90% cresol solution dermally showed widespread edema of the internal organs, especially the brain and kidney (Green, 1975). The liver showed signs of centrilobular and midzonal necrosis.

Chronic systemic poisoning by any route of exposure may produce symptoms of vomiting, dysphagia, salivation, diarrhea, loss of appetite, headache, fainting, dizziness, and mental disturbances (Sittig, 1981). Skin rash and discoloration may also result from prolonged or repeated exposure of the skin. Death may result from severe damage to the liver and kidneys. Oral poisoning has resulted in kidney problems (likely from the direct action of cresol) and pancreatitis (from constriction of the pancreatic ducts) (Klimkiewicz *et al.*, 1974, as reported in HSDB, 1995).

V. Effects of Exposures to Animals

The effects of inhaled o-cresol were examined in several species (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992 and U.S. EPA, 1982). Cats exposed for 30 minutes to 5-9 mg o-cresol/m^3 showed signs of respiratory irritation as indicated by increased parotid gland secretions. Exposure of mice for 2 hrs/day for 1 month to 50 mg o-cresol/m^3 did not have an effect on mortality, however, heart muscle degeneration and degeneration of nerve cells and glial elements were observed.

Uzhdavini *et al.*(1972) exposed rats (both sexes, numbers not stated) by inhalation to 9.0 ± 0.9 mg o-cresol/m^3, first for 2 months (6 hours/day, 5 days/week), then for 2 more months (4 hours/day, 5 days/week). Endpoints examined in rats included elementary conditioned defensive reflex, white blood cell levels, bone marrow elements, and liver function (as indicated by increased susceptibility to hexobarbital narcosis). Both cresol-exposed and control animals showed some loss of the defensive reflex, with the effect occurring in all exposed animals before the end of the second month and in control animals at later times. White blood cell counts were elevated in male animals, peaking at the end of the exposure period and returning to normal one month after cessation of exposure. Exposed animals also showed a statistically significant change in the leukoid-to-erythroid ratio in the bone marrow. Liver toxicity was suggested by an extension of hexobarbital narcosis duration in treated animals. Although guinea pigs were similarly evaluated for changes in blood cell counts and ECG, scant reporting of experimental detail limits the usefulness of this portion of the study.

NR rats were exposed by inhalation to 0.0052 or 0.05 mg tricresol/m^3 for 3 months (Kurliandskii *et al.*, 1975; as described by U.S. EPA, 1982). The proportional composition of the compound was not specified. Effects observed in the high-dose group included decreased weight gain, increased central nervous system excitability, increased oxygen consumption, and histological changes in the lung and liver. Serum gamma-globulin levels were also reduced. No effects were observed in the low-dose group. Rats (6/group, sex unspecified) were also exposed for 24 hours to 0.01, 0.1, and 2.4 mg tricresol/m^3 with a control group of 6 rats for each exposure group. The absorption of neutral red dye by lung tissue was used as an indicator of protein denaturation in the tissue. Significantly increased dye absorption over control animals was observed at both 2.4 and 0.1 mg tricresol/m^3. No significant increase in dye absorption over controls was observed in the low-dose group. The degree of dye absorption in the low-dose group, however, was higher than that observed at 0.1 mg tricresol/m^3.

Dermal exposure of rats to 1.0-1.7 ml cresol/kg body weight for 1-2 hours resulted in skin discoloration and death of the animals (Campbell, 1941).

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Uzhdavini *et al.*, 1972
Study population	Rats
Exposure method	Discontinuous inhalation exposure
Critical effects	Alterations in bone marrow cellularity
LOAEL	9 mg o-cresol/m^3
NOAEL	Not observed
Exposure continuity	average 5 hours/day, 5 days/week
Exposure duration	4 months
Average experimental exposure	1.3 mg/m^3 for LOAEL group
Human equivalent concentration	1.3 mg/m^3 for LOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h))
LOAEL uncertainty factor	10
Subchronic uncertainty factor	1
Interspecies uncertainty factor	3
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	300
Inhalation reference exposure level	0.004 mg/m^3 (4 µg/m^3; 0.001 ppm; 1 ppb)

The available literature on the observed toxicity of cresol compounds and cresol mixtures to humans indicates that these compounds are primarily toxic from their ability to cause chemical burns and are therefore of concern at the site of contact. In humans, inhalation exposure is reported to cause respiratory effects including the development of pneumonia, pulmonary edema, and hemorrhage (Clayton and Clayton, 1982). Other case reports of cresol toxicity to humans are confounded by the presence of other compounds, such as phenol, formaldehyde, and ammonia (Corcos, 1939; NIOSH, 1974). The only quantitative information from inhalation exposures to humans, however, comes from acute exposure studies showing irritation in 8 of 10 individuals exposed to 6 mg cresol/m^3 (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992). Toxic effects reported in the literature of cresols to animals include bone marrow and liver toxicity in rats from 4 month exposure to 9 mg cresol/m^3 (Uzhdavini *et al.*, 1972, as reported in U.S. EPA, 1982). Other animal studies have shown more systemic effects from inhalation exposure to cresols, including cardiac and nerve cell degeneration in mice exposed for 2 hour/day for 1 month to 50 mg o-cresol/m^3 (Uzhdavini *et al.*, 1972) and decreased weight gain with histological changes in the liver and lungs of rats exposed for 3 months to 0.05 mg tricresol/m^3 (Kurliandskii *et al.*, 1975, as reported in HSDB, 1995). Although this study reports adverse effects at levels below those observed in the Uzhdavini *et al.* (1972) study, limited experimental detail precludes the use of these data in the development of the chronic REL. The observation of lung injury (as indicated by increased dye uptake in the tissue) in rats exposed to 0.1 mg tricresol/m^3 for 24 hours is of limited use because of non-uniform results in exposed and control animals and the lack of specificity of this method in identifying lung injury (Kurliandskii *et al.*, 1975, as reported in HSDB, 1995).

The only useful data for the development of a chronic REL are those showing hematological toxicity to the bone marrow of rats exposed for 4 months to o-cresol (Uzhdavini *et al.*, 1972, as reported in U.S. EPA, 1982). These authors report a LOAEL of 9 mg tricresol/m^3. An uncertainty factor of 10 was incorporated to account for the absence of a NOAEL and standard uncertainty factors of 10 were applied to account for interspecies extrapolation and potentially sensitive human subpopulations.

As noted above, the study conducted by Kurliandskii *et al.* (1975) suggests that adverse health effects occur in experimental animals at exposure levels considerably below those reported by Uzhdavini *et al.* (1972) (9 mg/m^3 vs. 0.05 mg/m^3). Although the report from which the lower level is drawn has limitations, it suggests that the uncertainty applied in the derivation of the chronic REL from the Uzhdavini *et al.* (1972) study is warranted. Furthermore, human subjects exposed briefly to levels below the LOAEL reported respiratory irritation, also suggesting that this REL is not overprotective of potential adverse health effects associated with chronic exposure to cresol.

The strengths of the inhalation REL include the use of measured inhalation exposure data of animals exposed over a significant fraction of their lifetime. Major areas of uncertainty are the lack of human data, the lack of observation of a NOAEL, and the lack of reproductive and developmental toxicity studies.

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